

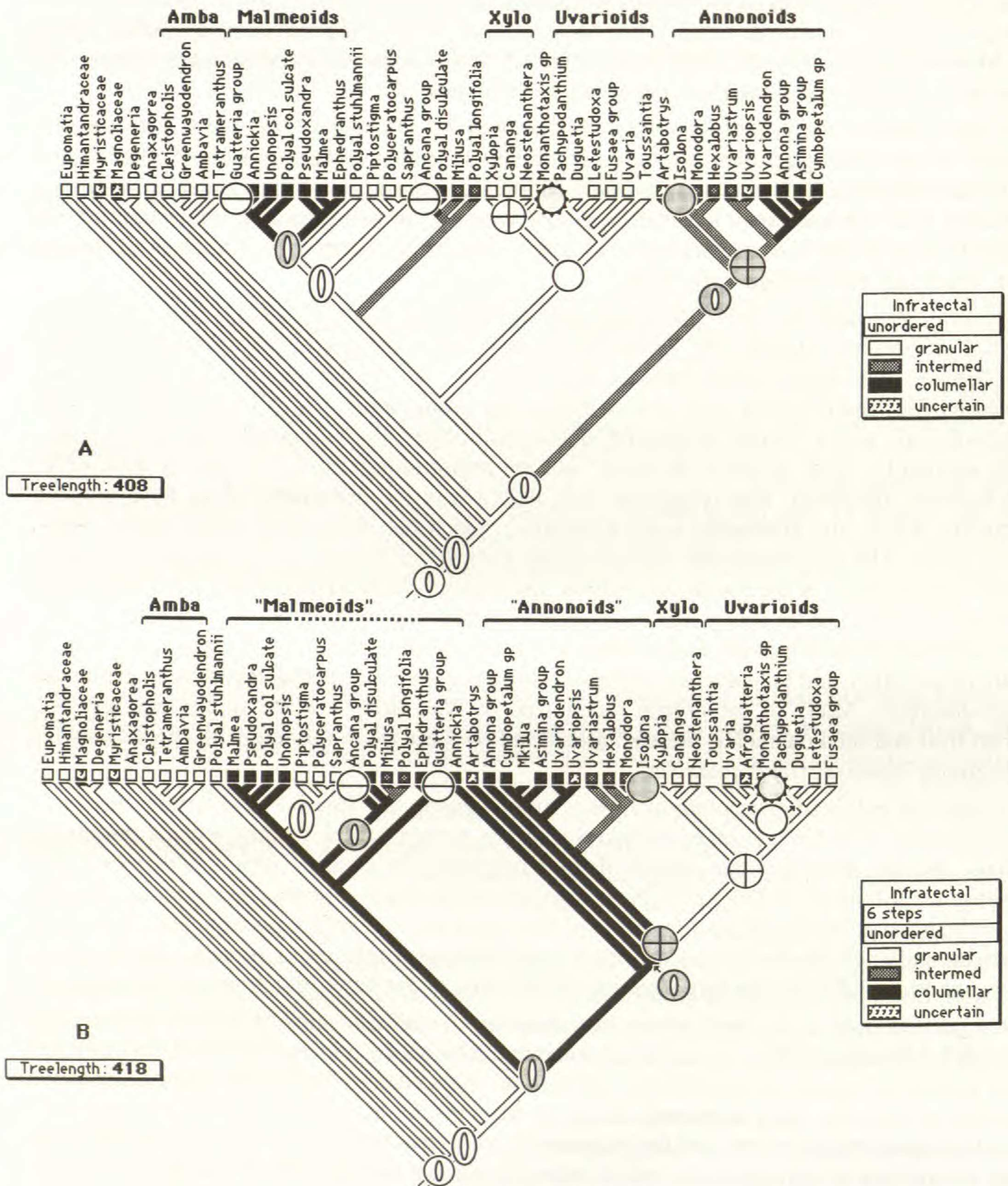


Fig. 1. — Representative most parsimonious trees from previous analyses, with sketches of pollen types superimposed at the point where they originate on a cladogram showing evolution of infratectal structure: **A**, DOYLE & LE THOMAS (1994); **B**, DOYLE & LE THOMAS (1995). Abbreviations: Amba = ambavioids, Xylo = xylopioids.

The present analysis has involved more substantial reinterpretation of characters and taxa. For example, reconsideration of floral characters in light of VAN HEUSDEN (1992) led us to reformulate our previous character for floral types (inspired by MORAWETZ 1988) and to replace number of monocarps in the fruit stage with number of carpels in the flower. In some cases where we scored taxa as uncertain because they show more than one state, new data on internal relationships allowed us to estimate the basic state, notably in the *Cymbopetalum* group, analyzed cladistically by JOHNSON & MURRAY (1995). Following MORAWETZ (1988), we originally combined *Ancana*, *Fitzalania*, and *Haplostichanthus* as the *Ancana* group, but because the unity of this group was questioned by VAN HEUSDEN (1992), we replaced it with *Ancana* alone.

In a companion article (DOYLE & LE THOMAS in press), we use this data set for an empirical test of the value of palynology. Pollen characters are most important in rooting the family: when they are removed from the data set, the basal groups are annonoids with columellar tetrads, rather than *Anaxagorea*. Consistency indices imply that pollen, fruit, and seed characters are generally less homoplastic than vegetative, floral, and inflorescence characters. Most recently, molecular analyses by VAN ZUILEN (1996) and BYGRAVE & CHASE (pers. comm.) have provided independent support for the view that pollen gives the correct rooting of the family. Although these studies differ in the genes studied, taxa included, and detailed results, they both confirm the basal position of *Anaxagorea* and the conclusion that *Artabotrys* and the groups with tetrads and inaperturate single grains form a clade.

The present article has three main objectives. The first is detailed justification of character definitions and documentation of scoring of taxa, including illustrations of leaf architectural characters. The second is exploration of the relative robustness of the various results using decay analysis and comparisons with slightly less parsimonious trees. The third is critical examination of the implications of the results for evolution not only of pollen characters, stressed in our previous articles, but also of all others. Because character evolution is the main focus of this paper, we present definition and documentation of characters and discussion of their evolution in the text, as done in an article with similar goals by TUCKER et al. (1993), rather than relegating the former topics to an appendix. In describing trees, we summarize the character support for major clades, but we devote less space to the relationships of genera, since these have been emphasized elsewhere (LE THOMAS et al. 1994; DOYLE & LE THOMAS 1995; LE THOMAS & DOYLE 1996), and in most cases our conclusions have remained similar despite changes in higher-level topology.

DATA AND METHODS OF ANALYSIS

Taxa

In order to root the Annonaceae, we included five other taxa of Magnoliales, namely Magnoliaceae, Myristicaceae, *Degeneria*, Himantandraceae (*Galbulimima*), and *Eupomatia*, which were the closest relatives of Annonaceae in the morphological analysis of primitive angiosperms by DONOGHUE & DOYLE (1989). We omitted Canellaceae, since this group was basal in

Magnoliales in some most parsimonious trees but associated with Winteraceae and Illiciales in others. Magnoliales as thus defined were either the sister group of all other angiosperms or a paraphyletic basal grade. The unity of the six families of Magnoliales and the association of Canellaceae with Winteraceae have been confirmed by analyses of *rbcL* sequences (CHASE et al. 1993). We rooted the whole tree with a hypothetical ancestor (list of ancestral states) based on Winteraceae, Canellaceae, and *Austrobaileya*, which were at or near the base of other clades of woody magnoliids, and outgroups to angiosperms as a whole found in analyses of seed plants by DOYLE & DONOGHUE (1986, 1992), namely Bennettitales, Gnetales, and *Caytonia*. This ancestor has been removed from the figures. When these outgroups show more than one of the states found in Magnoliales, we scored the hypothetical ancestor as uncertain (e.g., 0/1). This reasoning may need modification in light of analyses of rDNA and revised morphological data (DOYLE et al. 1994; DOYLE 1996), which indicate that angiosperms are rooted among “paleoherbs”, with Nymphaeales basal, and Magnoliales are nested within a more advanced clade of woody magnoliids. In this case non-angiospermous groups would be too distant to be relevant. However, considering non-angiosperms had little effect except in scoring the ancestral states for pollen shape, tectum, and infratectal structure as uncertain, whereas other woody magnoliids would imply they were globose, reticulate, and columellar, respectively.

A recurrent problem in selecting and scoring taxa is internal variation in states (often called polymorphism). There are two general solutions to this problem. In the exemplar approach, one member is chosen to represent the group, ideally one that is relatively plesiomorphic, which the analysis would place where it would place the common ancestor of the group, or the whole group if all its members were included. In the compartmentalization approach, the group is treated as a unit and an attempt is made to reconstruct the ancestral states of variable characters, based on information on relationships within the group. Unambiguous scoring is possible when the same ancestral state can be reconstructed in both basal branches, or the state in one is uncertain, but when ancestral states in the two branches differ, the group must be scored as uncertain.

There has been much recent discussion of the relative merits of these two approaches (e.g., NIXON & DAVIS 1991; MISHLER 1994; NIXON et al. 1994; DONOGHUE 1994). When there is no homoplasy, the increased number of unknown states involved in compartmentalization can lead to lower resolution, but when combined with homoplasy it can lead to positively incorrect relationships (NIXON & DAVIS 1991). Assumptions on relationships within groups can be incorrect; different results might be found if both ingroup and outgroup taxa were included in a global analysis. However, the exemplar approach is equally problematical (DONOGHUE 1994). It can also lead to misplacement of groups when the exemplars have convergences with other groups that were not present in the common ancestor of the group they represent. Such errors might be avoided by compartmentalization, especially when relationships within the group are clear under all reasonable outgroup assumptions. The ideal solution is to split each group into all component monomorphic taxa, but with large groups this is impossible from a practical standpoint.

In general, we have adopted a pragmatic compromise between these approaches. We treated some groups that all authors have considered natural (FRIES 1959; WALKER 1971a; MORAWETZ 1986a, 1986b; VAN SETTEN & KOEK-NOORMAN 1992; VAN HEUSDEN 1992) as single taxa: *Annona* group (*Annona*, *Anonidium*, *Raimondia*, *Rollinia*, *Rolliniopsis*: pseudosyncarpy, $n = 7$), *Asimina* group (*Asimina*, *Deeringothamnus*: no pedicel articulation), *Cymbopetalum* group

(*Cymbopetalum*, *Bocagea*, *Cardiopetalum*, *Froesiodendron*, *Hornschuchia*, *Porcelia*, *Trigynaea*: no bracteoles, polyads), *Fusaea* group (*Fusaea*, *Duckeanthus*), *Guatteria* group (*Guatteria*, *Guatteriella*, *Guatteriopsis*, *Heteropetalum*: sulculate pollen, reduced exine, $n = 14$: MORAWETZ & WAHA 1985), and *Monanthotaxis* group (*Monanthotaxis*, including African species formerly assigned to *Popowia*, *Enneastemon*, *Exellia*, *Gilbertiella*: lianas, few stamens, echinate pollen). In the malmeoids, which are not assuredly monophyletic and hard to divide into subgroups, we used several exemplars chosen to illustrate distinctive character combinations (e.g., inflorescence position, bracteoles, sepal and petal estivation, ovule number). We split *Polyalthia* sensu lato into five groups chosen to represent the range of pollen morphology and to test whether the genus is monophyletic (as assumed by LE THOMAS 1980/81): *Greenwayodendron* (*P. suaveolens*, *P. oliveri*, segregated by VERDCOURT 1969), *P. stuhlmannii* (sulcate, granular), *P. longifolia* (round, reduced sulcus), disulculate *Polyalthia* group (types B and D of WAHA & HESSE 1988, including *P. jenkinsii*, *P. obliqua*, *P. rumphii*) and columellar-sulcate *Polyalthia* group (*P. hypoleuca* complex, Malagasy groups B and C: ROGSTAD & LE THOMAS 1989; SCHATZ & LE THOMAS 1990). The latter two groups are not definitely monophyletic, but we chose the species used in scoring them to be relatively homogeneous in the characters used. *Artabotrys* also varies in several characters (cf. CHRISTMANN 1987), but its monophyly is supported by its unique inflorescences modified into hooks for climbing. Each decision runs the risk of error, but we believe the risks are tolerable in an initial study of a group of this size, as long as it is recognized that the assumptions should be tested in the future.

Several changes from our previous treatments represent a tendency toward an exemplar approach and narrower taxon definitions that pose fewer problems of polymorphism. Since studies by CHATROU (1996) indicate that *Malmea* as previously delimited was heterogeneous, we rescored this genus to correspond to CHATROU's concept of *Malmea* sensu stricto (*M. obovata*, *M. dielsiana*, *M. guianensis*, etc.), with flat midrib, spiniform endosperm ruminations, and glassy endosperm. Replacement of the *Ancana* group with *Ancana* alone (following VAN HEUSDEN 1992) led us to rescore petal shape, fruit wall, and raphe, which were previously uncertain. We also eliminated *Friesodielsia* from the *Monanthotaxis* group, since its placement here was questioned by VAN HEUSDEN (1992), but this did not lead to any change in scoring (supporting the view that *Friesodielsia* belongs here after all). As thus defined, the *Annona*, *Asimina*, *Guatteria*, *Fusaea*, and *Monanthotaxis* groups could probably have been replaced by the genera after which they are named, since these genera are plesiomorphic in most or all features. We have continued to define them as groups, but for the sake of conciseness we will usually refer to them as *Annona*, *Asimina*, *Guatteria*, *Fusaea*, and *Monanthotaxis*. An exception is the *Cymbopetalum* group, which is more diverse and will be referred to as a group.

In other cases, we have retained a compartmentalization approach but improved it by reducing the number of uncertain scorings, based on new data on relationships within clades. Thus the cladistic analysis of the *Cymbopetalum* group (Bocageae) by JOHNSON & MURRAY (1995) indicated that this group consists of two clades, with *Froesiodendron* at the base of a line leading to *Porcelia*, *Cymbopetalum*, and *Cardiopetalum*, and *Trigynaea* the sister group of *Bocagea* and *Hornschuchia*. This allowed us to decide which states are basic in three characters previously scored as uncertain: oil cells in the palisade layer, small micropylar plug, and absence of idioblasts in the seed coat. On the other hand, following the conclusion of RAINER (1996, pers. comm.) that the

basal taxa in *Annona* are sections *Euannona* and/or *Atta*, and assuming that *Raimondia*, *Rollinia*, and *Rolliniopsis* may be nested within *Annona* (cf. SCHATZ & LE THOMAS 1993), we rescored stigma form in the *Annona* group as either elongate or capitate (as in *Atta*), rather than the former. Similarly, other members of the *Monanthotaxis* group differ from *Monanthotaxis* mostly in apomorphies and may be nested within the latter. In the outgroups, we assumed that *Liriodendron* is the sister group of other Magnoliaceae (cf. CHASE et al. 1993) and *Mauloutchia* and *Brochoneura* are basal in Myristicaceae, based on their relatively unfused stamens and granular pollen (WALKER & WALKER 1981).

Characters

Definitions of characters are presented in the section on character evolution, the data matrix in Table 1. We have distinguished between unknown (?) and uncertain (0/1) for binary characters, although these are equivalent in tree construction, in order to show the nature of the uncertainty.

We used 79 potentially informative characters (where the presumed derived state occurs in more than one taxon, i.e., excluding autapomorphies), 51 of which are binary, 28 multistate, requiring a minimum of 113 character state changes for the whole data set. Multistate characters are unordered, except in 10 cases where there is a clear logical basis for ordering (e.g., quantitative characters). Since justification of character definitions, choices among controversial interpretations, and sources of data are a major focus of this paper, they will be discussed primarily in the section on character definition and evolution, but some generalities are in order here. Recurrent problems include incomplete study (sampling) of characters across the family, presence of more than one state within taxa (already encountered in the discussion of taxa), and continuous characters. All of these represent weak points of the analysis and thus potential topics for future study.

We did not include several promising characters because data were missing for a majority of taxa (e.g., floral anatomy: DEROIN 1989; several embryological characters: STEINECKE 1993). However, we did retain some such characters that appear to be uniform in Annonaceae, since they bear on the monophyly of the family and/or its relationship with outgroups (e.g., nodal anatomy, phloem characters).

We eliminated other characters because they show too much variation within taxa (e.g., several leaf anatomical characters of VAN SETTEN & KOEK-NOORMAN 1986) — using them would require many uncertain scorings. However, in some such cases, two or more states recognized by previous authors tend to co-occur within taxa, but others do not; here we combined related states in ways that reduce the number of uncertain scorings. Examples include epidermal crystals (VAN SETTEN & KOEK-NOORMAN 1986), sieve tube plastids (BEHNKE 1988), inflorescence types (FRIES 1959), and endosperm consistency (VAN SETTEN & KOEK-NOORMAN 1992), all discussed below.

Some of the most difficult problems concerned continuous (quantitative) characters. In general, we defined states in terms of average rather than maximum numbers within species. We eliminated many characters because they seemed too continuous and/or too variable within taxa (e.g., several leaf architectural characters, noted below). However, when sufficient data were available, we tried to define limits between states at natural breaks in the distribution of measures (again, this helps reduce the number of polymorphisms within taxa). For example, average pollen sizes for

TABLE 1. — Data matrix used in this study. A = 0/1, B = 0/2, C = 1/2, D = 1/3, E = 2/3, F = 0/1/2, G = 0/1/3.

	1	2	3	4	5	6	7
	123456789012345678901234567890123456789012345678901234567890123456789						
Outg	A0?000000A???	00?00000AA?0AA?	0?0000000A00AA00AB0B0?	?????0B?A?1?A???	00A0?0A?0??		
Eupo	01011A00000???	00210011A0?C01?	??2???????	01000020100000002200?	??0?100????	0000011?100	
Hima	00001000120???	11101100101101?	??2???????	1100000010000001?	20111?100????	0000?00?000	
Dege	01?1100000???	101111001011001	100000000001000001	10000000020000?	0?100????	0010201?0??	
Myri	011010000C0???	10A0?10011?101?	02?????????	000000A0A011?	0030?0?000????	0C0000A?000	
Magn	0A11100A01?1?	10C01101A0110001	0A000000000000010B0C011	2020A000?000????	0010200?????		
Afro	11???	0110?????????	00?10010010000000020002010000201120D0?	1?1011101000???	3?????		
Amba	01???	0000?????????	111100?001100001001001000000010011010?	0?1011101100???	10000		
Anax	010010A001A0A11111?	1A111000001100002010000010000010012020?	??001110000A000C11100				
Anca	01???	0010?????????	101100100110001B002000201102011011100?	001011000000???	22100		
Anni	01???	021A?1?1111011?	1001100100110100200E000010202011012130?	1111121100000CE21???			
Anno	0110A011A1A011111111100110010011000020020111212020211?	2C310???	1A1C1000A0032E0100				
Arta	110000110?0?	1111111110112001001100012002000011C0C021011120?	001A1FA00A00AD231???				
Asim	0110011100001111011111001001001000000002011121202021121100?	00101100002001231???					
Cana	01?000220?0?	111011111111100100110001B003011121000100022200?	11111110002011211100				
Clei	01?000110?0?	11100111?1111000001000111002000000000110012010?	A1101C10010011210000				
Cymb	010?01100?00111101?	1?00100010A1A0000100?	11112120202?A11A00?	102A1C00002001232101			
Dugu	010?001010A1A111011110A1100110100000000200011110?	???	1?22312???	10110A00100?131101			
Ephe	010?0A2201110?????	10121000010000010020000102020111?	2130?1011100000000133????				
Fusa	01???	2A110210011101?110A110111010000001020111210000200?	22311???	1011000010????	31101		
Gree	01???	01A0?0???	1111???	1011110000110001000100000000010012010?	1011100101000132????		
Guat	010?201A0C01011001111102200100100000000200021A?0????	122130?1A1111AA00A0012E0110					
Hexa	01???	011020?111AA11?110110011011100?0002011111C01020011100?	001012A010000323????				
Isol	01???	01110?0???	11211?11101C001001?100?000200111110102001A102???	101210100003331???			
Lete	11???	0110?????????	00110111010000000020002111000200?	22311???	1A111000100113????		
Malm	010?00000011011101???	0A11000001000000002000010202011122130?	10111C0100A00132????				
Mili	01???	0011011?01110111?1A?20010011010000010002011010102021A0?	10111100000001332100				
Mkil	01???	0100?????????	00?1001001?00000002111121?0???	012100?0?2112?00020????	3????		
Mona	11???	0220?0?A?1211?11AA1100100110000A002000101?10???	ACCA00?101111A0100003231???				
Mono	01?001110?A?111?11111A0110010011101?1002011111201021B1A102???	101C10100003131???					
Neos	01???	0220?0?0?1101???	0011001001100112003111111000100012230?	11111110100003231???			
Pach	01???	20C01?0?0110111?1A111001101000001002000101?1???	1?22312???	10110000100123????			
Pipt	01???	0120?????	1101???	1111001001101012002000010000000121100?0?	10110100000132????		
Post	01???	0000?????	10?1001001100000002000000000010112130?	101111?00000????	2?????		
Polo	01?000110?1???	1?????	1111001001100010002000001101010111130?	10111100000000332100			
Pocs	01?0?0000???	0?1???	110110010011000A000200001020201112C130?	10111201000001222100			
Podi	01???	001A0?1???	1???	1?10?10010011000A00020002011020111121G0?	10111A0A0000013E2100		
Poly	01???	0220?0?????	2?????	1011101001100000002000010000110121100?00101A1100000112????			
Pseu	01???	01000201111111???	11ACC0000010000010020100102020111121G0?	1011110100000332????			
Sapr	01???	00220210011201?1?	0011001001000000002000211000000122100?00101C00000001332???				
Tetr	00???	201111001110111???	10220000010000000010000010000201C1010?0?	10121001001?210010			
Tous	11???	0210?????	101100111A000000002011111?0???	2?212000?101111100010????	3?????		
Unon	010021110201011101???	11110010011000010020000102020111C21G0?	101112A100A002222???				
Uvar	11000011110?0112011110A110A1AA1000000A02000111000020AC2A00?	101A1C101A1003231100					
Uvas	01???	00100?????	11111???	1A11001111100000002011111201021011000?0010121010000323????			
Uvad	01???	0110?????	101200A001100000002011111202021122100?001011A010000323????				
Uvop	01???	0000?1?11???	1?11A11A0?00110000000201111120C02A202000?00101100100003231000				
Xylo	0100001A0000A110011111111C00100110001211E11111100010002C200?A0?A111000C001C31101						

genera based on WALKER (1971a) tend to cluster below, between, or above limits of 45 and 90 μm . A problem is that these measures are averages of sizes in several component species, whereas it would be theoretically preferable to reconstruct the ancestral size for the taxon as a whole. Some may argue that such characters should not be used in phylogenetic analysis, but often they do appear to characterize groups, and the extremes are so different that it seems a loss of information to eliminate them completely.

Since our first analyses, it has been clear that some characters are worthless at the systematic level under consideration, in the sense that they originate independently everywhere they occur (e.g., unisexual flowers). This is not to say that they are worthless at all levels — they may help unite the members of taxa in which they are found. Although it is tempting to remove such characters because of their effect on measures of homoplasy, we have not done so: first, because they pose interesting evolutionary problems that we wished to evaluate in the context of an analysis of the whole data set; and second, because we did not want to exclude *a priori* the possibility that they might prove to be informative with addition of taxa or changes in the interpretation of other characters.

Analyses

Data sets were analyzed with PAUP (SWOFFORD 1991). In searching for most parsimonious trees, we performed varying numbers of replicate analyses (usually 20-30) with stepwise random addition of taxa and TBR branch swapping. This increases the likelihood of finding different “islands” of most parsimonious trees (MADDISON 1991), within which other equally parsimonious trees can be found by swapping one taxon at a time, but between which more complex (and therefore inaccessible) rearrangements are required. We used the constraints option in PAUP (with constraint trees in which taxa in question are forced together as polychotomies) to investigate the relative parsimony of alternative hypotheses. We used MacClade (MADDISON & MADDISON 1992) to analyze character evolution and the implications of alternative arrangements. When we state that a clade is united by particular characters, these are characters that unambiguously change at that point on the tree, as indicated by MacClade.

Among techniques for investigating the relative support for different clades, bootstrap analysis (FELSENSTEIN 1985) was impractical because of the large number of taxa, but we did perform a decay analysis (BREMER 1988; DONOGHUE et al. 1992). This method determines the minimum number of extra steps required (how much parsimony must be relaxed) before trees are found in which a given clade breaks up. This is done by searching for all trees equal to or less than a given length, constructing a strict consensus of these trees, and observing which clades remain in the consensus. In searching for one step less parsimonious (“one-off”) trees (426 steps), we performed four analyses with stepwise random addition of taxa (for a total of 14 replicates), one of which was still not complete after reaching the memory limit of 12,000 trees. This was probably not an exhaustive search of trees of this length, but since all but one of the six clades remaining in the 12,000-tree analysis were found in all other analyses, we suspect that the conclusions on decay are complete. Eight or nine searches were performed for two-off (427 step) and three-off (428 step) trees, saving 5000 trees in each analysis. Although these searches are even less likely to be exhaustive, overlap in the consensus trees suggests that the conclusions are accurate.

The one-off trees obtained in the decay analysis were also valuable for insights into slightly less parsimonious alternative relationships and “families” of trees. Besides inspecting individual trees and the majority-rule consensus, we used the constraints option in PAUP to “filter” the trees obtained. For example, in order to understand why the basal position of *Anaxagorea* decays in the consensus of one-off trees, we filtered trees that *are not* consistent with a constraint tree in which

Anaxagorea is basal and all other taxa form a polychotomy. Other classes of alternative relationships were discovered by filtering trees that *are* consistent with constraint trees in which taxa form suspected alternative clades (e.g., those found in previous analyses), or by conducting a new search for most parsimonious trees with the same constraints.

PHYLOGENETIC RESULTS

Most parsimonious trees

Analysis of the complete data set yielded 180 most parsimonious trees of 425 steps. These trees belong to two islands (MADDISON 1991), island A consisting of 40 trees, island B of 140. The two islands are identical except in arrangements of taxa in the annonoid clade. Strict consensus trees of both islands are shown in Fig. 2.

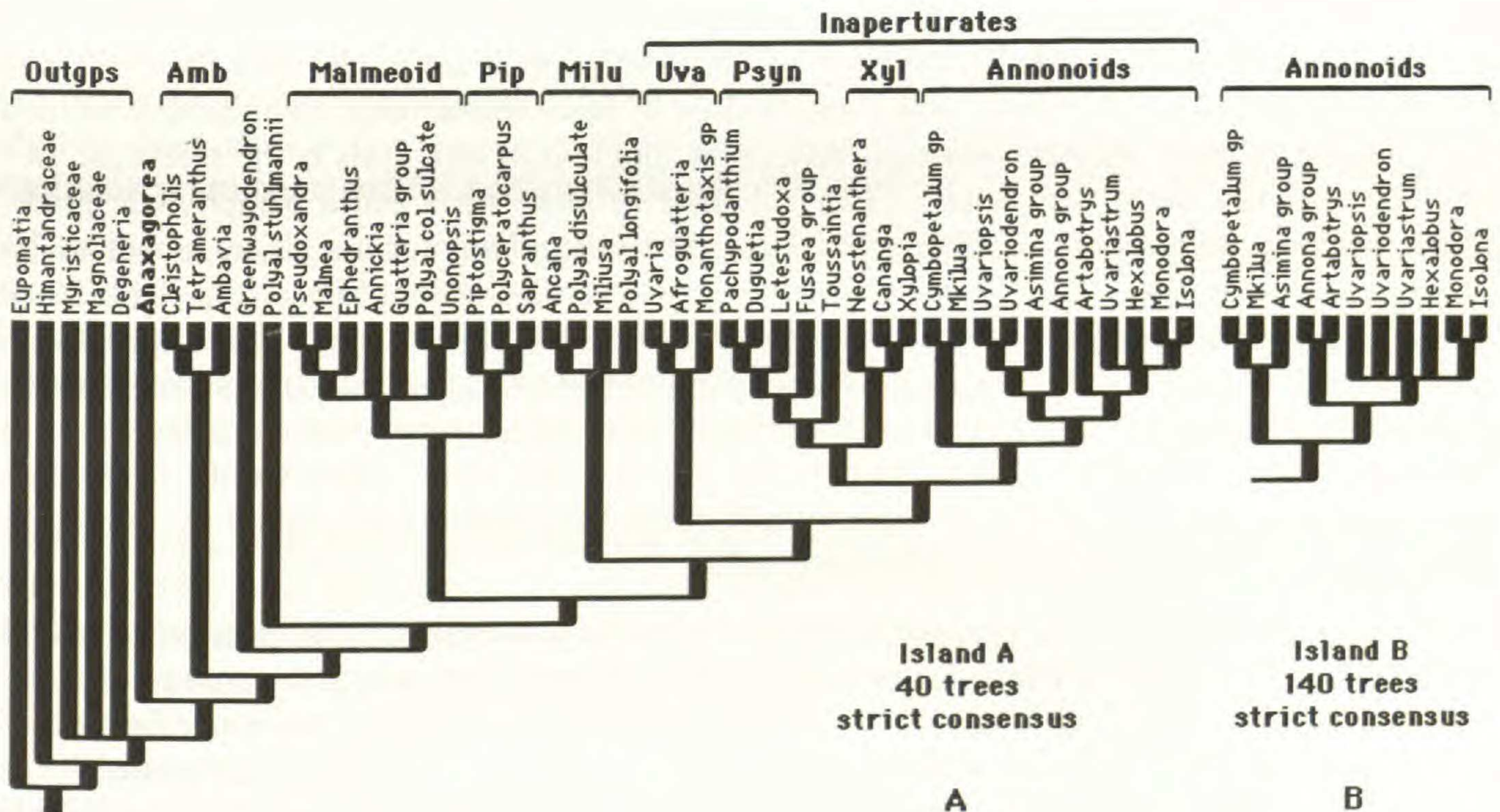


Fig. 2. — Consensuses of most parsimonious trees (425 steps) found in the present analysis: **A**, strict consensus of 40 trees in island A; **B**, differing portion of the strict consensus of the 140 trees in island B. Abbreviations: Outgps = magnolialian outgroups, Amb = ambavioids, Pip = piptostigmoids, Milu = miliusoids, Uva = uvarioids, Psyn = pseudosyncarps, Xyl = xylopioids.

As in our previous analyses (DOYLE & LE THOMAS 1994, 1995), outgroup relationships of Annonaceae are poorly resolved, but the three closest groups are always Magnoliaceae, Myristicaceae, and *Degeneria*. In one arrangement (not seen previously), Annonaceae are linked with Magnoliaceae based on isoquinoline alkaloids and imbricate sepals, and the two groups are linked with Myristicaceae by astrosclereids, 1-2 nexine foliations, and dehiscent fruit (retained in *Anaxagorea*). In other trees, Annonaceae are linked with Myristicaceae by cymes, in which case Magnoliaceae and *Degeneria* are united by multilacunar nodes, medium-sized pollen, sarcotesta, and idioblasts in the outer integument. Magnoliaceae plus *Degeneria* may be linked with Annonaceae by 1-2 corolla cycles (vs. sepals only). Magnoliaceae plus Myristicaceae (united by lateral prophylls and thickened outer foliation) may be linked with Annonaceae by astrosclereids and 1-2 foliations. Finally, the three outgroups may form a clade united by lateral prophylls and linked with Annonaceae by distinct (vs. calyptrate) sepals, boat-shaped pollen, and 1-2 foliations. Relationships within Annonaceae are remarkably insensitive to these variations in outgroups: the same set of ingroup trees is found in every case.

As in DOYLE & LE THOMAS (1994, 1995), the basal branch in Annonaceae is *Anaxagorea*, which retains several states found in other Magnoliales but lost in other Annonaceae: ventral plate of vascular tissue in the midrib, inner staminodes (equivocally primitive when the sister group is Magnoliaceae or Magnoliaceae plus Myristicaceae), laminar stamens, idioblasts in the inner integument, and (in some arrangements) dehiscent fruit.

The next four groups are *Ambavia*, *Tetrameranthus*, *Cleistopholis*, and *Greenwayodendron*, called “ambavioids” by DOYLE & LE THOMAS (1994, 1995), which are linked with “higher” groups by a simple arc of vascular tissue in the midrib, loss of inner staminodes, stamens that are narrower but have a tongue-shaped connective extension, and loss of idioblasts in the seed coat. In our previous analyses, these four genera formed a clade in some trees and a paraphyletic grade in others, but now only *Ambavia*, *Tetrameranthus*, and *Cleistopholis* form a clade, united by large micropylar plug and the chromosome number $n = 7$ (MORAWETZ 1986b; MORAWETZ & LE THOMAS 1988). *Greenwayodendron*, which retains prolonged stamens and a sessile stigma, is united with the remaining Annonaceae by stipitate monocarps, transverse mesotesta fibers, and spiniform endosperm ruminations; these characters overrule features that it shares with the ambavioids (two lateral ovules, tuberculate seeds). Hence we restrict the name **ambavioids** to *Ambavia*, *Tetrameranthus*, and *Cleistopholis*. *Tetrameranthus* and *Cleistopholis* are united by intermediate tertiary venation and a reversal to imbricate petals.

The next branch above *Greenwayodendron* is *Polyalthia stuhlmannii*, which differs from other *Polyalthia* groups and resembles *Greenwayodendron* in retaining granular monosulcate pollen. This position, also found in DOYLE & LE THOMAS (1995), was a change from our first analysis (DOYLE & LE THOMAS 1994), in which *P. stuhlmannii* was associated with the malmeoids. *P. stuhlmannii* resembles lower groups in having reticulate tertiary venation, but it is linked with higher groups by solitary flowers, valvate sepals, peltate-truncate stamens, globose-cylindrical receptacle, and capitate stigma. These advances establish the floral pattern that is basic for the majority of Annonaceae. As noted in DOYLE & LE THOMAS (1995), this arrangement implies that all higher Annonaceae were derived from an ancestor of a *Polyalthia* type, with small flowers and spreading, valvate petals.

Among the next groups, the **malmeoids** formed a clade in DOYLE & LE THOMAS (1994) but broke up in DOYLE & LE THOMAS (1995), with *Annickia* (= *Enantia*) associated with *Artabotrys* and the inaperturates, and with the piptostigmoids nested within the remaining “malmeoids” in some trees. In the present analysis, the malmeoids are again a monophyletic group, united by silica cells in the epidermis and characteristic pollen features: reticulate tectum, columellar infratectal layer, and thickened outer foliation. *Annickia* may be associated with *Ephedranthus*, *Malmea*, and *Pseudoxandra*, based on rhombic epidermal crystals, or interpolated between *Guatteria* and *Unonopsis* plus the columellar-sulcate *Polyalthia* group, in which case these four taxa are united by complex midrib histology and crossed mesotesta fibers. The columellar-sulcate *Polyalthia* group is united with *Unonopsis* by an increase in chromosome number to $n = 9$. *Ephedranthus*, *Malmea*, and *Pseudoxandra* are linked by imbricate sepals and petals (this is equivocal when *Annickia* is located above *Guatteria*, which also has imbricate petals). *Malmea* and *Pseudoxandra* are united by secondarily straight secondary and reticulate tertiary venation.

As in most of our previous trees, the malmeoids are linked to a clade consisting of *Piptostigma*, *Polyceratocarpus*, and *Sapranthus*, here designated the **piptostigmoids**, by osteosclereids, medium-sized pollen, and glass-like endosperm. The piptostigmoids are united by percurrent tertiary venation, secondarily sessile monocarps, and thick fruit wall. *Polyceratocarpus* and *Sapranthus* are linked by curved secondary veins and high vessel density.

The next clade, here named the **miliusoids**, consists of two *Polyalthia* groups, *Miliusa*, and *Ancana*, united by rhombic epidermal crystals, verrucate tectum, a shift from granular to intermediate infratectal structure, and an increase to $n = 9$. The basal group is either *Miliusa*, based on short petals, or *Polyalthia longifolia*, based on retention of a vestigial sulcus; the other taxa are united by disulculate pollen. *Ancana* and the disulculate *Polyalthia* group are united by columellar infratectal structure and thickening of the outer foliation.

As in our second analysis (DOYLE & LE THOMAS 1995; Fig. 1B), *Artabotrys* and the taxa with inaperturate tetrad pollen and single grains form a clade, here designated the **inaperturates** or the **inaperturate clade**, united by inaperturate pollen, multiple foliations, large micropylar plug, and crossed mesotesta fibers. This is very different from our first analysis (DOYLE & LE THOMAS 1994; Fig. 1A), in which these groups formed two adjacent lines much lower on the tree, just above the ambavioids.

Relationships within the inaperturate clade show significant changes from DOYLE & LE THOMAS (1995; Fig. 1B). First, in that analysis, the “annonoids” formed a basal paraphyletic group of two lines, one consisting of *Annona*, the other of the remaining taxa, implying that all the inaperturates were derived from an ancestor with columellar tetrads. Now *Annona* is nested among other taxa with columellar tetrads, so that the **annonoids** again form a monophyletic group. Second, the former “uvarioids” are split into two well-separated clades. One consists of *Uvaria*, *Afroguatteria*, and *Monanthotaxis*, which formed most of the “liana clade” of DOYLE & LE THOMAS (1995), to which we now restrict the term **uvarioids**, united by liana habit, high vessel density, oil cells in the endosperm, and idioblasts in the nucellus. The uvarioids, which retain single pollen grains, are the sister group of the remaining inaperturates, which are united by medium-sized tetrad pollen with reduced proximal exine. The other clade is the **pseudosyncarps**, linked with the African liana *Toussaintia* (previously associated with the liana clade based on habit, sessile stigma, and nume-

rous ovules) by large sepals, imbricate petals, and a rudimentary aril. The *Toussaintia*-pseudosyncarp clade is linked with the **xylopioids** by elongate stigma.

These shifts in ingroup relationships are correlated with changes in outgroups. In DOYLE & LE THOMAS (1994), *Artabotrys* was the sister group of the annonoid tetrads, and in DOYLE & LE THOMAS (1995) it was the sister group of the whole inaperturate clade, based on globose pollen with multiple foliations, and the two were linked with *Annickia*. However, in the present trees *Annickia* is again nested within the malmeoids, whereas *Artabotrys* is nested within the annonoids. The sister group of the inaperturates is the miliusoids, based on a shift from boat-shaped to globose pollen and from spiniform to lamelliform endosperm ruminations.

Within most of the inaperturate groups, changes in topology and character support are minor. The xylopioids are united by elongate petals with a concave base (a new synapomorphy reflecting revision of the floral type character), peltate-apiculate stamens, fused basal infratectal granules, and loss of nexine foliations. *Cananga* and *Xylopia* are united by low vessel density, cymes, concave receptacle apex, and bilobed aril. The pseudosyncarps are united by one basal ovule, pseudosyncarpy, and thick fruit wall. Taxa above *Fusaea* are united by secondarily single pollen, normal proximal exine, and verrucate tectum; *Duguetia* and *Pachypodanthium*, by secondarily reticulate tertiary venation, stellate-peltate hairs, globose-cylindrical receptacle, and basal ring of sterile carpels in the fruit. In the uvarioids, *Monanthotaxis* is basal rather than *Uvaria*; however, *Afroguatteria* still acts as a link between the two in having small pollen like *Monanthotaxis* (a symplesiomorphy) and imbricate petals (a synapomorphy with *Uvaria*). Like *Uvaria*, it lacks the exine reduction, tectal spines, and other autapomorphies of *Monanthotaxis* (curved secondary veins, percurrent tertiaries, wide rays).

Greater changes are seen in the annonoids. This group is now united by idioblasts in the palisade parenchyma, reticulate tectum, and columellar infratectal structure — states that were basic in the inaperturate clade and homologous with *Annickia* in DOYLE & LE THOMAS (1995). Relationships within the annonoids appear to have been destabilized by rescoring the chromosome number of *Asimina* as $n = 8$ (MORAWETZ pers. comm.) and stigma of *Annona* as either capitate or elongate, which allows it to be nested within this predominantly capitate group, and replacement of the floral type character. *Artabotrys* is linked with *Annona* in island B (Fig. 2B), based on basally concave petals, but with *Uvariastrum*, *Hexalobus*, and the parasyncarpous genera *Monodora* and *Isolona* in island A (Fig. 2A), based on reduced carpel number. *Asimina* is linked with *Mkilua* and the *Cymbopetalum* group in island B, as in our previous analyses, based on large pollen, reduced micropylar plug, and bilobed aril, but with *Uvariopsis* and *Uvarioidendron* in island A, based on axillary inflorescences and reduced micropylar plug, and the three are linked with *Annona* by lateral prophylls and globose-cylindrical receptacle. The line leading to the *Cymbopetalum* group is the sister group of other annonoids; primitive states that support this position are absence of idioblasts in the seed coat, thin fruit wall (island A), and narrow rays (island B). *Mkilua* and the *Cymbopetalum* group are united by secondarily reticulate tertiary veins, locellate anthers, dorsal fruit dehiscence, and (in island A) bilobed aril. *Uvariastrum*, *Hexalobus*, and the parasyncarps always form a clade in island A, united by axillary inflorescences and oil cells in the endosperm; *Hexalobus* may be linked with the parasyncarps, based on petal fusion, or with *Uvariastrum*, based on large sepals. In island B, *Uvariastrum*, *Hexalobus*, and the parasyncarps

are associated with *Uvariopsis* and *Uvariadendron* in various arrangements, some of which will be mentioned below.

Support statistics and alternative relationships

The many changes from one analysis to the next reflect the high level of homoplasy in Annonaceae (cf. DOYLE & LE THOMAS 1994). An indication is the consistency index (CI) (an inverse measure of homoplasy), 0.27, which is below average for this number of taxa (0.36: SANDERSON & DONOGHUE 1989). As a result, different trees and scenarios for character evolution can be expected with addition or reinterpretation of characters and taxa.

One measure of the relative support for various relationships is the number of characters that change unequivocally at each node (Fig. 3). This does not include cases in which it is equally parsimonious to assume that an apomorphy arose once at the base of the clade and was reversed within it or arose twice within the clade. This criterion implies that some of the more robust clades (supported by four or more unequivocal synapomorphies) are the Annonaceae as a whole, the groups above *Anaxagorea*, the groups above *Greenwayodendron*, the malmeoids, the miliusoids, the

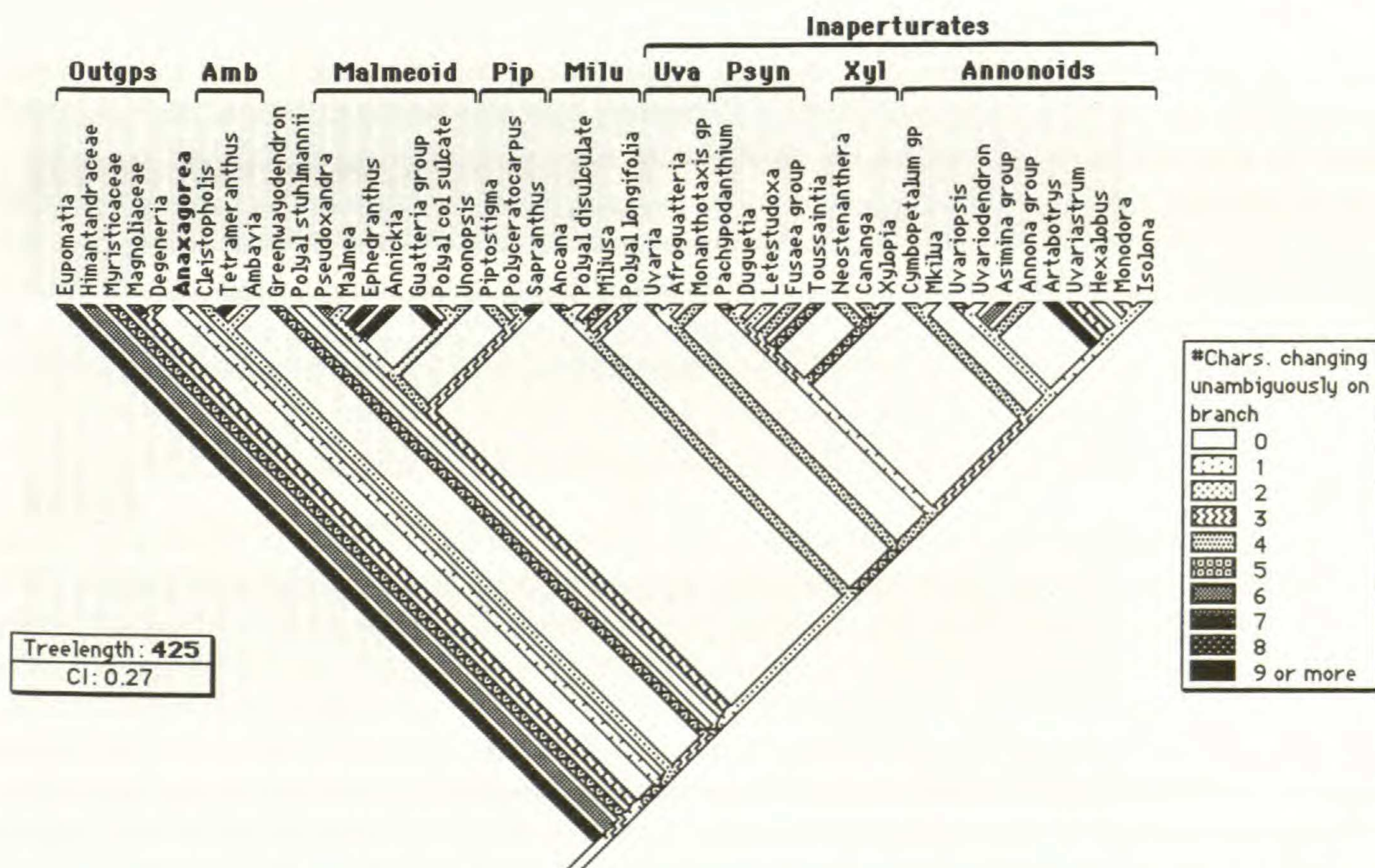


Fig. 3. — Representative most parsimonious tree from island A, showing number of characters that change unequivocally at each node.

the inaperturates, the uvarioids, the annonoids (in island B), *Mkilua* plus the *Cymbopetalum* group (in island A), the xylopioids, *Cananga* plus *Xylopia*, and *Duguetia* plus *Pachypodanthium*.

An important consideration is the fact that changes in individual terminal taxa are homoplasies with other groups, since we did not include autapomorphies in the matrix. Terminal taxa with many changes are therefore groups whose position may be unstable, although this is not necessarily so if their various autapomorphies are convergences with several different taxa. Taxa with five or more autapomorphies are *Tetrameranthus*, *Greenwayodendron*, *Pseudoxandra*, *Ephedranthus*, *Annickia*, *Guatteria*, *Sapranthus*, *Ancana*, *Miliusa*, *Pachypodanthium*, *Toussaintia* (in island A), *Uvariopsis* (in island A), *Asimina* (in island A), and *Artabotrys*. As seen above, some of these groups (*Annickia*, *Uvariopsis*, *Asimina*, *Artabotrys*) do indeed “jump” to different positions even in different equally parsimonious trees. Alternative positions of *Guatteria*, *Sapranthus*, *Ancana*, and *Toussaintia* will be noted in the discussion of one step less parsimonious trees.

The problem with number of changes as a measure of support is the fact that it does not take into account the distribution of homoplasy. For example, there may be an almost equal number of changes in other characters indicating that one member of a group belongs elsewhere. This problem is addressed by methods such as bootstrap and decay analysis.

Results of the decay analysis (BREMER 1988; DONOGHUE et al. 1992; Fig. 4) illustrate the nearly equal parsimony of different major arrangements. In the consensus of most parsimonious and one-off trees (less than or equal to 426 steps), the only clades that remain are Annonaceae as a whole, the uvarioids, xylopioids, pseudosyncarps, and the *Duguetia-Pachypodanthium* group. Thus the basal position of *Anaxagorea*, the unity of the groups above *Greenwayodendron*, and the ambavioid, malmeoid, piptostigmoid, miliusoid, inaperturate, and annonoid clades are no longer seen in some one-off trees. In two-off trees (427 steps), only the Annonaceae and the xylopioids remain. In three-off trees (428 steps), the Annonaceae also decay, leaving only the xylopioids.

As is generally the case, this exercise obscures the amount of structure in trees of a given length, since many of the polychotomies in the consensus trees are due to “jumping” of single taxa between two (almost) equally parsimonious positions and changes in the rooting of groups that otherwise remain intact. This expectation is confirmed by inspection of the one-off trees, aided by filtering for particular alternatives with the constraints option, and by searching for trees in which taxa are constrained to form different clades. The relationships revealed in this way could become more parsimonious with new data, and their recognition helps in identifying groups in need of further study.

Decay of the outgroup relationships is due to the existence of one-off trees in which *Eupomatia* is the sister group of Annonaceae, a frequently proposed alternative hypothesis (CORNER 1976; BEHNKE 1988; MORAWETZ 1988). This relationship is supported by wide rays, Pcs type sieve tube plastids (restricted to the two groups), isoquinoline alkaloids (also found in Magnoliaceae), a change from the *Tetrameranthus*-type to prochromosomal nucleotype, and limited *rbcL* sequence data (CHASE et al. 1993). Except that the arrangement of ambavioids, *Greenwayodendron*, and higher groups is unresolved, relationships within Annonaceae are unaffected, reaffirming the insensitivity of ingroup topology to assumptions about outgroups. The decay of Annonaceae in three steps is due to the existence of trees in which Magnoliaceae are inserted just above *Anaxagorea*.

Breakdown of the basal position of *Anaxagorea* also involves less radical changes than it appears. In 69% of the trees in which *Anaxagorea* is not the sister group of other Annonaceae, the

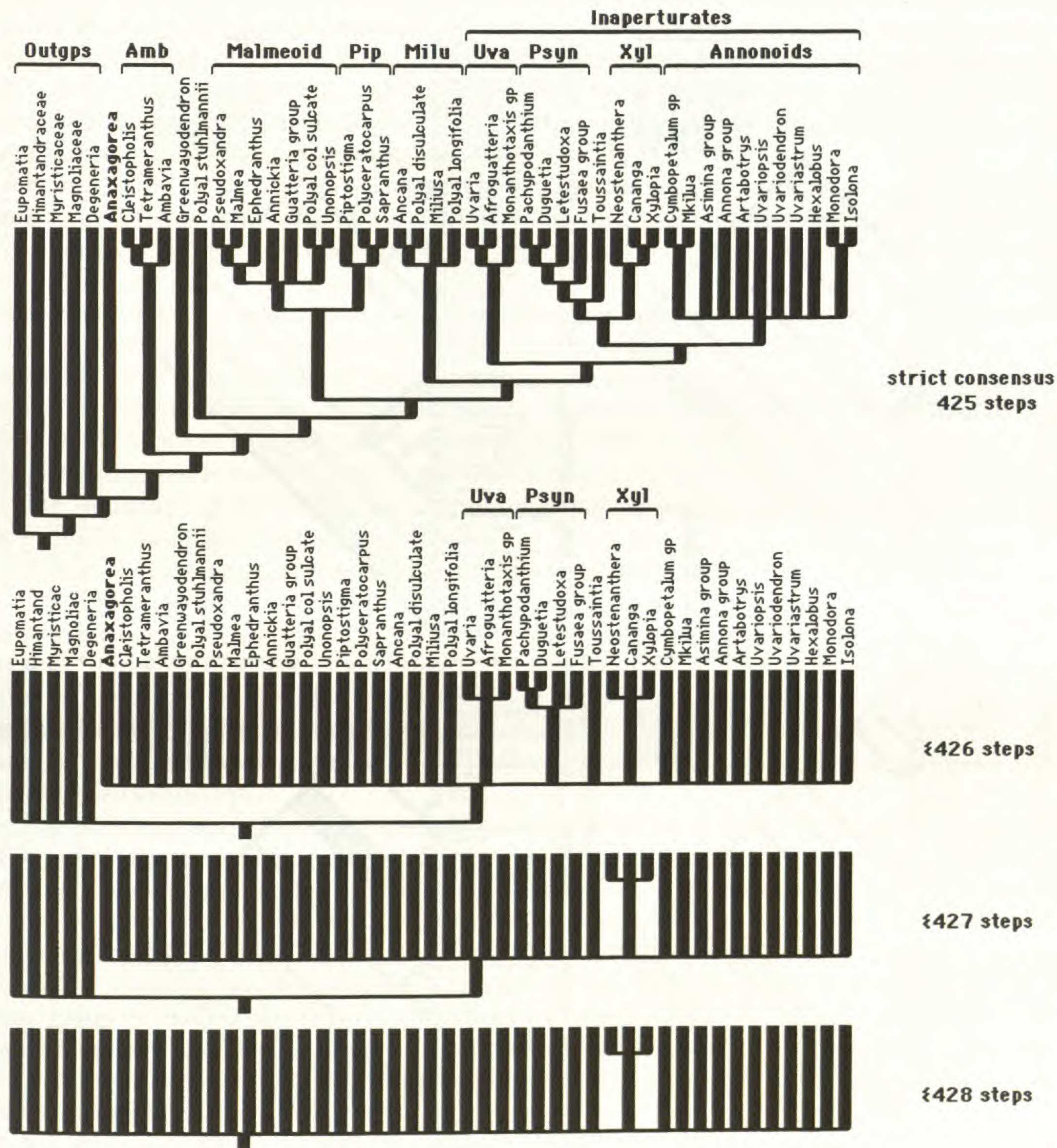


Fig. 4. — Decay analysis (BREMER 1988), showing strict consensus of trees of lengths equal to or less than the number of steps indicated.

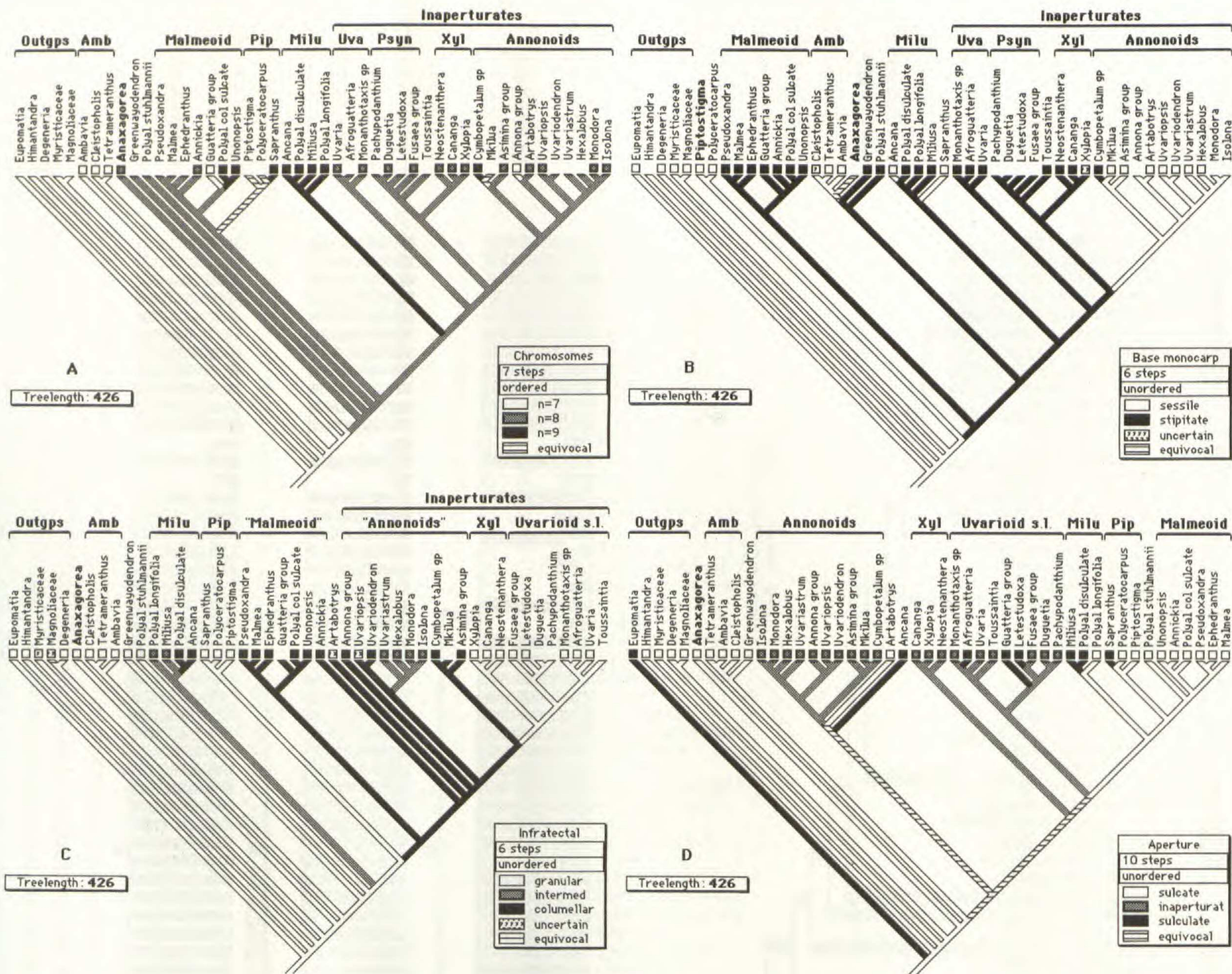


Fig. 5. — Representative one step less parsimonious trees (426 steps): **A**, with ambavioids rather than *Anaxagorea* basal in Annonaceae, showing evolution of chromosome number; **B**, with *Piptostigma* and *Polyceratocarpus* basal, showing evolution of monocarp base; **C**, with *Annickia* and *Artabotrys* associated with the inaperturate clade (as in DOYLE & LE THOMAS 1995), showing evolution of infratectal structure; **D**, with annonoids, xylopioids, and uvarioids sensu lato forming a paraphyletic series below miliusoids, piptostigmoids, and malmeoids (somewhat as in DOYLE & LE THOMAS 1994), showing evolution of pollen aperture.

ambavioids are basal, constituting either a clade or two lines, and in 50% *Anaxagorea* is the next branch — a simple reversal of the two most basal groups. These trees still give a fairly consistent picture of primitive states in Annonaceae (e.g., low-rank leaves, prolonged stamens, granular monosulcate pollen, thick endosperm laminations). Synapomorphies that would unite *Anaxagorea* with more advanced groups are a shift from thick to thin fruit wall and from the *Tetrameranthus*-type to prochromosomal nucleotype, and, when the ambavioids form two lines, from an original chromosome number $n = 7$ to $n = 8$ (Fig. 5A).

Other groups in the basal polychotomy of trees in which *Anaxagorea* is not basal are *Greenwayodendron* and *Polyalthia stuhlmannii*, the next two branches in the shortest trees; the piptostigmoids, at the base of the line leading to the malmeoids; the malmeoids; the miliusoids; and the inaperturates. This does not mean that all of these groups can be basal. For example, *Piptostigma* and *Polyceratocarpus* (with granular monosulcate pollen, considered primitive by LE THOMAS 1980/81) may be the sister group of other Annonaceae (Fig. 5B), but not the whole piptostigmoid clade. When these two genera are basal, the third genus, *Sapranthus*, is linked with the miliusoids, based on rhombic epidermal crystals, disulculate pollen, and $n = 9$. This relationship, proposed by MORAWETZ (1988) and WAHA & HESSE (1988), is also found in some one-off trees in which *Anaxagorea* is basal. Together, the dissociation of the piptostigmoids from the malmeoids and the “jumping” of *Sapranthus* to the miliusoids pull the malmeoids, miliusoids, and inaperturates into the basal polychotomy.

Other such rearrangements explain the breakdown of other clades. For example, some 426-step trees are of the type found in DOYLE & LE THOMAS (1995), with *Annickia* and *Artabotrys* associated with the inaperturates (Fig. 5C). Since *Annickia* is nested within the malmeoids in the shortest trees, its “jumping” to this next-best position destroys the unity of the malmeoids. Similarly, “jumping” of *Artabotrys* from within the annonoids to a lower position breaks up the annonoids and disrupts the arrangement of other inaperturate clades. In these trees the uvarioid clade also reappears in its original broad sense, with *Toussaintia* associated with the uvarioids sensu stricto rather than the pseudosyncarps.

Other 426-step trees (Fig. 5D) represent a variation on those found in DOYLE & LE THOMAS (1994), in which annonoids, xylopioids, and uvarioids sensu lato form a paraphyletic series below the miliusoids, piptostigmoids, and malmeoids. This draws the various inaperturate lines into the basal polychotomy of one-off trees. In these trees, *Ancana* is the sister group of *Artabotrys* and the annonoids, based on columellar exine structure, thickened outer foliation, and low carpel number. *Guatteria* “jumps” from the malmeoids to a very different position in the uvarioids sensu lato, as sister group of the pseudosyncarps, supported by imbricate petals, rudimentary aril, complex midrib histology, and one ovule per carpel. Other one-off trees (not illustrated) show a third position of *Sapranthus*, as sister group of the pseudosyncarps, based on osteosclereids, terminal inflorescences, and imbricate petals.

CHARACTER DEFINITION AND EVOLUTION

In this section, we first present definitions of characters and sources of data, grouped into related categories, then discuss problems encountered in interpretation of characters and implications of our results for their evolution. The following references were consulted throughout and will not

be cited individually, except in cases of conflict or special interest. Conventional vegetative and floral characters: SINCLAIR (1955), FRIES (1959), LE THOMAS (1969), VERDCOURT (1971), HUBER (1985), and the synoptic key of MAAS et al. (1983), supplemented by our own observations on herbarium and preserved material at Paris, USNM, Berkeley, and Davis; *Afroguatteria*: BOUTIQUE (1951), PAIVA (1966); *Ambavia*: LE THOMAS (1972); *Anaxagorea*: MAAS & WESTRA (1984), HESSE et al. (1985); *Ancana*: MORAWETZ (1988), our observations on ENDRESS material; *Polyalthia* and putative relatives: ROGSTAD (1989), ROGSTAD & LE THOMAS (1989), SCHATZ & LE THOMAS (1990), ENDRESS material of *P. longifolia*; *Sapranthus*: SCHATZ (1987); *Tetrameranthus*: WESTRA (1985), MORAWETZ (1986b); *Austrobaileya*: ENDRESS (1980); *Degeneria*: BAILEY & SMITH (1942), SWAMY (1949), ENDRESS material; Himantandraceae: BAILEY et al. (1943), ENDRESS (1977); Magnoliaceae: NOOTEBOOM (1985).

Unless otherwise indicated, multistate characters are unordered.

Habit and leaf-stem transition

1. Habit (0) trees or shrubs, (1) lianas or lianescent shrubs or trees.
2. Phyllotaxy (0) spiral, (1) distichous at least on horizontal branches (BENZING 1967; MAAS et al. 1983).
3. Prophylls (0) adaxial, (1) lateral (FRIES 1959, in several cases verified by our observations; P.F. STEVENS pers. comm. for *Eupomatia*, Himantandraceae, Myristicaceae, *Anaxagorea*, *Asimina*).
4. Nodes (0) trilacunar, (1) multilacunar.
5. Histology of midrib (0) simple arc, (1) arc plus adaxial plate, (2) complex, with phloem and/or sclerenchyma intruding xylem body.
- 4, 5: JOVET-AST (1942), KAVATHEKÁR & PILLAI (1976), SUGIYAMA (1979), METCALFE (1987), VAN SETTEN & KOEK-NOORMAN (1986).

If the derived state included only lianas, the habit character would be uninformative, since the only exclusive liana is *Letestudoxa*. In DOYLE & LE THOMAS (1994), we scored *Uvaria* as uncertain, since FRIES (1959) described it as including trees, shrubs, and lianas. However, all the species described by SINCLAIR (1955) and LE THOMAS (1969) are at least lianescent. Some Malagasy species are described as trees by CAVACO & KERAUDREN (1958) and on herbarium labels at Paris, but only a small minority. Since *Uvaria* may grow for some time as a tree before becoming lianescent, DOYLE & LE THOMAS (1995) rescored it as lianescent. Our first analysis (DOYLE & LE THOMAS 1994) implied that lianescence arose independently everywhere it occurs, but in DOYLE & LE THOMAS (1995) it became one of the advances uniting the liana clade of the uvarioids, with only two other origins, in *Letestudoxa* and *Artabotrys*. *Toussaintia* now appears to be an independent origin, but lianescence is still a synapomorphy of the uvarioid clade in its restricted sense.

Distichous phyllotaxy is basic in Annonaceae and homologous with the same state in other Magnoliales (except Himantandraceae and most Magnoliaceae). The one exception, the spiral phyllotaxy of *Tetrameranthus*, is an autapomorphy, like its tetramerous perianth.

Although prophylls are lateral in Magnoliaceae and Myristicaceae, our results indicate that adaxial prophylls, as in *Eupomatia* and Himantandraceae, are basic in Annonaceae. Origin of lateral prophylls is a synapomorphy of *Annona* and *Asimina* in trees of island A, but a convergence in island B. These results are very weak, since data are lacking in other annonoids, except *Artabotrys* and the *Cymbopetalum* group, as well as most other Annonaceae. Satisfactory understanding of this character will require many more observations of living material.

Nodal anatomy has been studied in only a few Annonaceae, but all of these are trilacunar, implying that the character is potentially useful in establishing the monophyly and/or external relationships of the family. Our results imply that trilacunar nodes are homologous with those in the outgroups; the multilacunar nodes of *Degenera*, Magnoliaceae, and *Eupomatia* are derived two or three times from trilacunar.

Midrib histology supports the basal position of *Anaxagorea*, the only member of Annonaceae that retains the adaxial plate characteristic of other Magnoliales. The rest of family is united by origin of a simple arc of vascular tissue. The complex type of VAN SETTEN & KOEK-NOORMAN (1986), roughly equivalent to the *Melodorum* type of JOVET-AST (1942), is a synapomorphy of *Unonopsis* and *Guatteria* (though *Annickia* and the columellar-sulcate *Polyalthia* group are unknown), an equivocal synapomorphy of the pseudosyncarps (found in *Fusaea* and *Pachypodanthium* but not *Duguetia*), and an autapomorphy in *Tetrameranthus*.

Leaf architecture

6. Midrib (0) concave or flat on adaxial side, (1) convex (HUBER 1985; VAN SETTEN & KOEK-NOORMAN 1986; our observations).

7. Secondary veins (0) straight or recurved, (1) moderately curved, (2) strongly curved (ordered).

8. Tertiary venation (0) reticulate, frequent intersecondary veins, (1) partially percurrent (toward margin), some intersecondaries, (2) strongly percurrent, no intersecondaries (ordered).

7, 8: based primarily on leaf X-rays provided by S.L. WING (Smithsonian Institution) and herbarium material at Paris, Berkeley, and Davis. We also consulted KLUCKING (1986), but because of the large number of apparent misidentifications, we were careful to verify all his observations.

We examined several other leaf architectural characters but concluded that they were too variable within taxa (i.e., leaf shape, angle and spacing of secondary veins, distance between secondary loops and the margin, outer secondary loops).

The midrib is primitively concave or flat but becomes raised in several lines. This change unites the parasyncarps (*Monodora* and *Isolona*) and possibly *Asimina* and the *Cymbopetalum* group in island B (*Mkilua* has a concave midrib), but it occurs independently in *Pseudoxandra* and *Unonopsis*.

Despite a high level of homoplasy, our results generally support HICKEY's (1977) concept of "increase in rank", or increasing regularity of successively higher vein orders. This is expressed most directly in tertiary venation, which ranges from reticulate with frequent intersecondary veins to strongly percurrent, but also to some extent in course of the secondary veins, from straight to strongly curved. Examples are illustrated in Fig. 6. These two characters are partially but not completely correlated. Illustrated exceptions include *Piptostigma* (Fig. 6D), *Annickia* (Fig. 6E), and *Duguetia* (Fig. 6F); others can be seen by comparing the two parts of Fig. 7.

Our results indicate that Annonaceae originally had reticulate tertiary venation, like other Magnoliales; this is retained in *Anaxagorea* (Fig. 6A), *Ambavia*, *Greenwayodendron*, and *Polyalthia stuhlmannii*. Because of variation among these groups, it is uncertain whether the basic state in secondary venation was straight or intermediate. The shift to intermediate tertiaries (Fig. 6B) is a synapomorphy of groups above *P. stuhlmannii* and also unites *Tetrameranthus* and

Cleistopholis. The piptostigmoids are united by a further advance to strongly percurrent tertiaries (Fig. 6D), *Polyceratocarpus* and *Sapranthus* by strongly curved secondaries as well. *Neostenanthera* and *Cananga* also have percurrent tertiaries, but it is equivocal whether this is a synapomorphy of the xylopioids as a whole, since *Xylopioides* is reticulate or intermediate. Parallel advances occur in *Ephedranthus* and *Monanthotaxis* (Fig. 6C). A shift to strongly curved secondaries without change in the tertiaries occurs in *Annickia* (Fig. 6E; equivocally homologous with *Ephedranthus*) and *Toussaintia* (equivocally homologous with the xylopioids). However, reversals to reticulate tertiaries are frequent, and they unite *Malmea* and *Pseudoxandra*, *Duguetia* and *Pachypodanthium*, *Mkilua* and the *Cymbopetalum* group, and sometimes *Uvariopsis* and *Uvariastrum* (Fig. 7), besides occurring in the columellar-sulcate *Polyalthia* group. The reversal in *Duguetia-Pachypodanthium* is not correlated with a change in the secondaries (Fig. 6F), whereas *Ancana* shows a reversal in its secondaries but not its tertiaries.

Leaf anatomy

9. Hairs (0) simple or absent, (1) stellate or peltate.
10. Sclereids (0) absent, (1) astrosclereids, (2) osteosclereids.
11. Epidermal crystals (0) druses or absent, (1) solitary rhombic, etc.
12. Silica idioblasts (0) absent, (1) present.
13. Oil cells (0) in sponge parenchyma only, (1) in palisade or both palisade and sponge parenchyma.

9-13: BEYER (1902), JOVET-AST (1942), RAO & WEE (1966), PATEL (1971), VAN SETTEN & KOEK-NOORMAN (1986), METCALFE (1987), ROGSTAD (1989).

VAN SETTEN & KOEK-NOORMAN (1986) noted that features such as sclereids and epidermal crystals do not occur in all specimens of genera in which they are recorded. Since much of this variation is presumably a function of maturity or environment, we scored taxa in which a given feature occurs in at least some specimens as having the feature.

We combined stellate and peltate hairs, since the latter intergrade with stellate hairs within *Duguetia*, and tufts of simple hairs (*Tetrameranthus*, some *Annona* species: VAN SETTEN & KOEK-NOORMAN 1986). Our results imply that stellate-peltate hairs are a synapomorphy of *Duguetia* and *Pachypodanthium* but arose independently in *Uvaria*, *Annona*, and *Tetrameranthus* (thus in retrospect tufts might be better considered a separate state).

Foliar sclereids show a high level of homoplasy. Astrosclereids appear to be basic in Annonaceae and homologous with those in Myristicaceae and Magnoliaceae. Their replacement by osteosclereids is a synapomorphy of the piptostigmoid-malmeoid line, with a reversal in *Ephedranthus* and loss of sclereids in *Malmea*. However, the situation is confused above the uvarioids, since the rest of the inaperturate clade includes taxa with all three states, and the basal state may be either astrosclereids or none; furthermore, the sampling is so incomplete that evolutionary scenarios would be unwarranted.

VAN SETTEN & KOEK-NOORMAN (1986) recognized several types of epidermal crystals, but most types other than rhombic crystals occur in single taxa or in association with other types, while druses alternate with no crystals and were not recorded separately. The most consistent distinction is between solitary crystals (usually rhombic) and druses or no crystals, a character documented

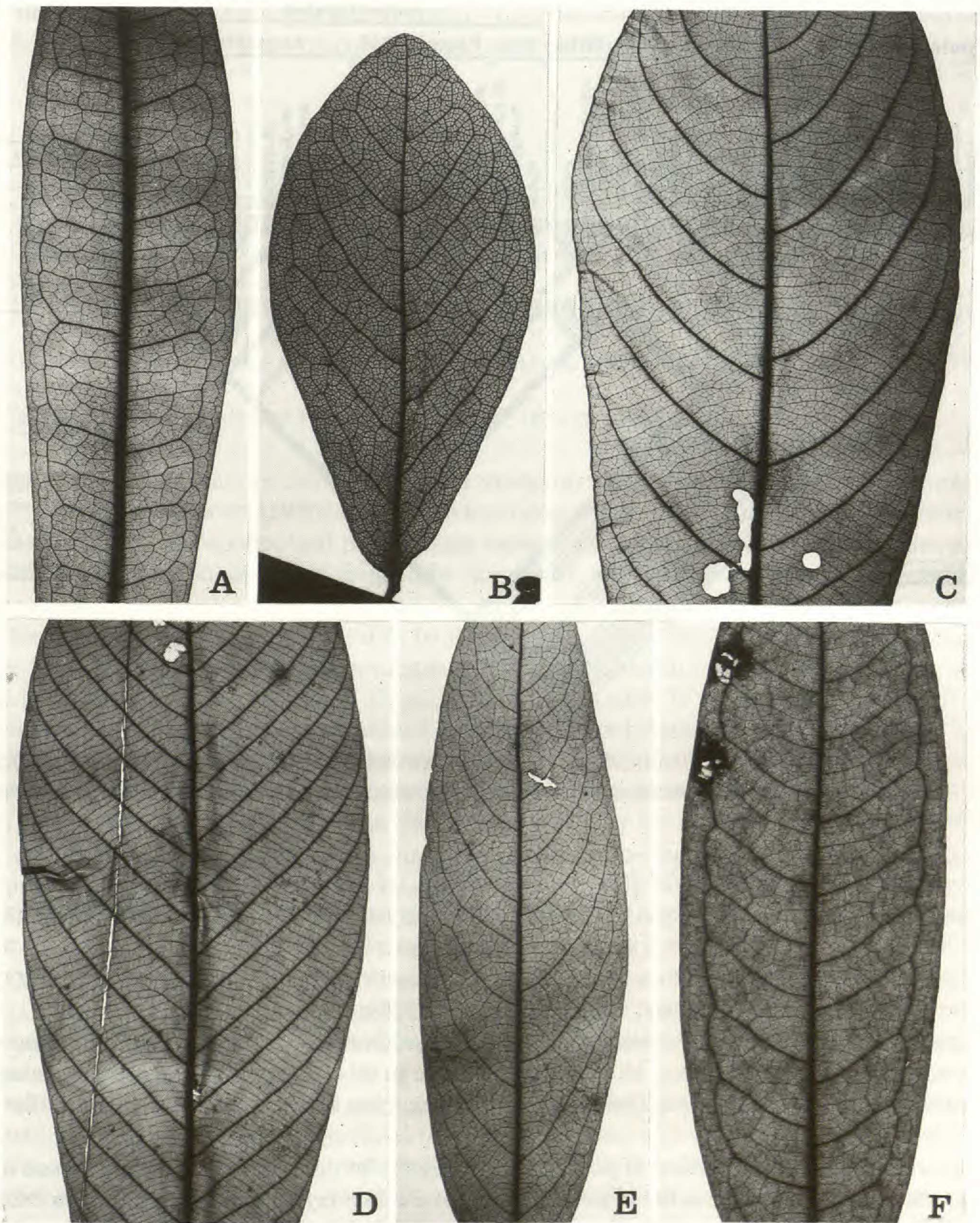


Fig. 6. — Leaf architectural characters in Annonaceae (X-rays, US), $\times 1$. — *Anaxagorea acuminata* (Dunal) A. DC.: A, straight secondary veins, reticulate tertiary venation. — *Asimina obovata* Nash: B, moderately curved secondary veins, intermediate tertiary venation. — *Monanthotaxis schweinfurthii* (Engl. & Diels) var. *seretii* (De Wild.) Verdc.: C, strongly curved secondary veins, percurrent tertiary venation. — *Piptostigma multinervium* Engl. & Diels: D, moderately curved secondaries, strongly percurrent tertiaries. — *Annickia chlorantha* (Oliver) Van Setten & Maas: E, strongly curved secondaries, intermediate tertiaries. — *Duguetia cauliflora* R.E. Fries: F, moderately curved secondaries, reticulate tertiaries.

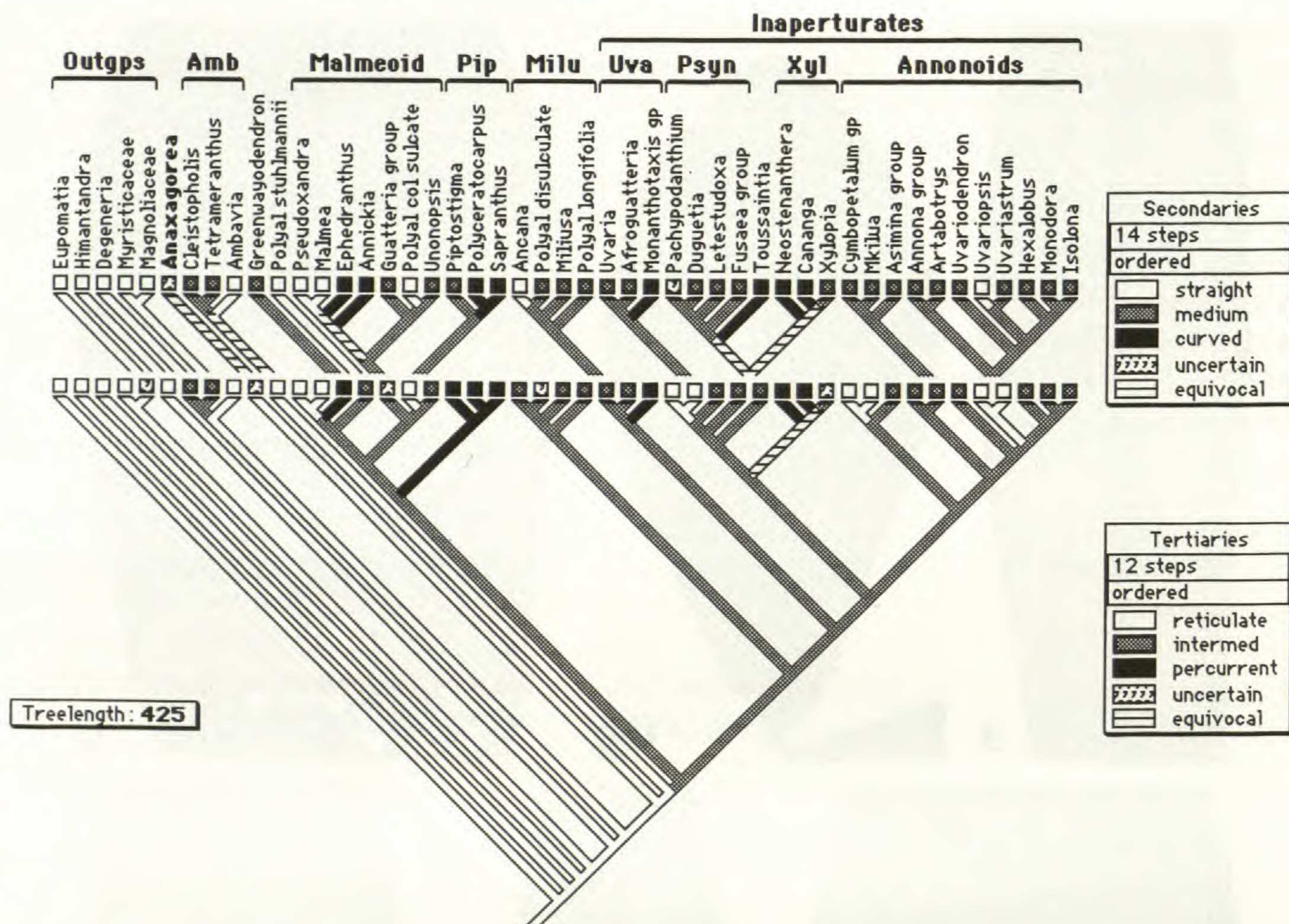


Fig. 7. — Representative most parsimonious tree (island B), showing evolution of secondary (above) and tertiary venation.

in paleotropical groups by JOVET-AST (1942). Solitary crystals are a synapomorphy of the miliuoids; in the malmeoids, they are a synapomorphy of *Annickia*, *Malmea*, and *Ephedranthus* when these groups are associated, with a reversal in *Pseudoxandra*. However, they are autapomorphic in *Sapranthus*, *Fusaea*, *Uvariopsis*, and several polymorphic taxa.

Silica cells are an important synapomorphy of the malmeoids, including *Guatteria*; the only independent origin is in *Duguetia*. However, confidence in this character is lowered by the lack of data outside the neotropics; in particular, the state is unknown in *Annickia* and the columellar-sulcate *Polyalthia* group.

The most consistent distinction in position of oil cells (idioblasts) is between occurrence in the sponge parenchyma only (or at the boundary between the two layers), and occurrence in the palisade parenchyma or (more commonly) in both layers (VAN SETTEN & KOEK-NOORMAN 1986). Since *Cleistopholis* and *Tetrameranthus* have oil cells in the palisade layer, but conditions in *Anaxagorea* are mixed, and the next groups studied have the opposite state, the basic state is equivocal. However, presence in the sponge layer only is basic for the malmeoids, piptostigmoids, miliuoids, uvarioids, xylopioids, and pseudosyncarps, with independent shifts to the palisade

layer in *Pseudoxandra*, *Annickia*, and *Cananga* (plus possibly *Xylopia*, which varies), and extension to the palisade layer is a synapomorphy of the annonoids.

Stem anatomy

14. Pith (0) normal, (1) septate.

15. Vessel perforations (0) scalariform, (1) simple.

16. Vessel density (0) $<10/\text{mm}^2$, (1) $10\text{--}40/\text{mm}^2$, (2) $>40/\text{mm}^2$ (ordered).

17. Rays (0) narrow, (1) wide (widest $>100\ \mu\text{m}$).

18. Parenchyma (0) diffuse or paratracheal, (1) apotracheal.

19. Phloem (0) normal, (1) stratified.

14–19: BEYER (1902), BAILEY & SMITH (1942), NORMAND (1950), VANDER WYK & CANRIGHT (1956), METCALFE (1987).

20. Sieve tube plastids (0) S or Psc type, (1) Pcs type (BEHNKE 1988).

Simple vessel perforations are an important synapomorphy of Annonaceae, as recognized by VANDER WYK & CANRIGHT (1956), with an independent origin in Himantandraceae. Septate pith, stratified phloem, and apotracheal parenchyma unite Annonaceae and other Magnoliales except *Eupomatia*; they contradict the frequently suggested relationship between *Eupomatia* and Annonaceae.

Vessel density and ray width appear to be the only potentially informative wood characters for higher-level relationships within Annonaceae, but like other continuous characters they are hard to break into cladistically useful states. In vessel density, VANDER WYK & CANRIGHT (1956) recognized five categories, which we combined into three ordered states. This defined a large “average” category, on the assumption that extreme densities may be most informative, and avoided scoring the *Guatteria* group (*Guatteria* few, *Heteropetalum* moderately few) as uncertain. For ray width, we adopted the limit of $100\ \mu\text{m}$ for average width of the broadest rays used by VANDER WYK & CANRIGHT, although statistical study might reveal a better separation. For other taxa, we measured these characters on plates in BAILEY & SMITH (1942), NORMAND (1950), and METCALFE (1987). VANDER WYK & CANRIGHT listed *Monodora* as having very numerous vessels, but the figure in NORMAND implies that it has moderately few; we therefore scored it as unknown. VANDER WYK & CANRIGHT listed *Hexalobus* as having moderately numerous vessels and narrow rays, but NORMAND shows moderately few vessels and wide rays; we scored both characters as (0/1). Since VANDER WYK & CANRIGHT did not indicate species of *Polyalthia* studied, we scored *Polyalthia* groups as unknown.

Vessel density has some value in uniting small groups. The basic vessel density in the family is intermediate, as in most other Magnoliales (except *Eupomatia*, with numerous vessels). An increase in vessel density is a synapomorphy of *Sapranthus* and *Polyceratocarpus* and of the uvarioids, besides occurring independently in *Isolona* (or *Isolona* plus *Monodora*?). A decrease unites *Tetrameranthus* and *Cleistopholis* (*Ambavia* is unknown) and *Xylopia* and *Cananga*, but is autapomorphic in *Guatteria* and *Pachypodanthium*.

Ray width is much more homoplastic. The ancestral state is equivocal because of variation among outgroups and basal Annonaceae, but narrow rays are basic for the groups above *Polyalthia stuhlmannii*. In island B, wide rays unite the annonoids above the *Asimina*-*Mkilua*-*Cymbopetalum*

clade, but this is equivocal in island A, where *Asimina* (with narrow rays) is nested within this group. However, wide rays also originate several times in single taxa (*Pseudoxandra*, *Monanthotaxis*, *Pachypodanthium*, *Cananga*).

S, Psc, and Pcs sieve tube plastid types form a continuum. We combined S and Psc because they intergrade in Magnoliaceae and Myristicaceae, and S is found by itself only in Himantandraceae. The only Magnoliales with Pcs are *Eupomatia* and Annonaceae, and this has been taken as evidence of relationship; however, our results imply that it is convergence.

Chemistry

21. Isoquinoline alkaloids (0) absent, (1) present (LEBOEUF et al. 1982).

Isoquinoline alkaloids are a potential synapomorphy of Annonaceae, Magnoliaceae, and/or *Eupomatia*; our results indicate that they may be homologous in Magnoliaceae (depending on out-group relationships) but convergent in *Eupomatia*. A great diversity of alkaloids has been reported in Annonaceae, but the literature is difficult to use cladistically, since only the presence of particular compounds is indicated, not their absence.

Inflorescences

22. Inflorescence position (0) terminal (leaf-opposed, supra-axillary, extra-axillary), (1) axillary.

23. Flowers (0) solitary (-2), (1) in cymes.

24. Articulation of pedicel (0) absent, (1) (sub)basal, (2) distal.

25. Bracteoles (0) absent, (1) 1-3 per flower, (2) >3 (ordered).

FRIES (1959), MAAS et al. (1983), WEBERLING (1988), our observations.

We considered a character for cauliflory, a conspicuous feature of many Annonaceae. However, only one taxon is exclusively cauliflorous, *Piptostigma*. In mixed groups (e.g., *Uvariadendron*), we based our scoring of other inflorescence characters on the non-cauliflorous members, since cauliflorous inflorescences are so condensed that their morphology is difficult to interpret.

All inflorescence characters appear to be highly homoplastic.

In inflorescence position, we distinguished basically terminal (including leaf-opposed, supra-axillary, and extra-axillary) and axillary. We previously scored the disulculate *Polyalthia* group as terminal, but data of SINCLAIR (1955) indicate it is axillary. Our results indicate that axillary inflorescences, as in most of the outgroups except some Magnoliaceae and *Eupomatia bennettii* (WEBERLING 1988), are ancestral. There are independent shifts to terminal in *Greenwayodendron*, *Malmea*, *Annickia*, and *Sapranthus*. In island A, a shift to terminal inflorescences is a synapomorphy of the inaperturates, while reversals to axillary unite several derived clades: *Cananga* and *Xylopia*; *Asimina*, *Uvariadendron*, and *Uvariopsis*; *Uvariastrum*, *Hexalobus*, and the parasyncarps. However, this is equivocal in island B.

A conventional distinction in Annonaceae is between solitary (or occasionally paired) flowers and cymes. However, as emphasized by CHATROU (pers. comm.), this distinction is problematical,

since all taxa with bracteoles on the pedicel (i.e., except the *Cymbopetalum* group) potentially have cymes, and the number of flowers may simply reflect the stage in development. This still leaves a somewhat less satisfactory distinction between groups that usually have only one flower at a time and those that have more. As thus defined, cymes appear to be basic in Annonaceae; they are homologous with those of Myristicaceae when the two families are sister groups, equivocally so with other outgroup arrangements. Cymes revert to solitary flowers above *Greenwayodendron*, and independently in *Tetrameranthus*. Above *Greenwayodendron*, reappearance of cymes is a synapomorphy of *Xylopia* and *Cananga* and occurs independently in *Unonopsis*, *Piptostigma*, *Polyalthia longifolia*, *Pachypodanthium* (plus possibly *Duguetia*, which is mixed), and *Artabotrys* (associated with a unique modification of the cymes into hook-like structures for climbing).

Other inflorescence characters are completely uninformative at this level of analysis, since the derived states arise independently everywhere they occur. Articulation of the pedicel, which is originally (sub)basal, becomes distal in *Tetrameranthus* and *Guatteria*, and is lost in *Asimina*. MAAS et al. (1983) listed *Asimina* as lacking bracteoles but noted that its cataphylls may appear bract-like; we do not distinguish these from bracteoles. Bracteoles become numerous in *Tetrameranthus*, *Ephedranthus*, *Guatteria*, *Miliusa*, *Artabotrys*, and *Uvarioidendron*, and are lost in the *Cymbopetalum* group.

Sex distribution

26. Flowers (0) bisexual, (1) male and bisexual or unisexual.

We combined production of male and bisexual flowers (androdioecy: *Greenwayodendron*, *Ephedranthus*, *Polyceratocarpus*) with unisexual flowers (Myristicaceae, *Uvariopsis*), on the assumption that the former condition may be a step toward the latter. We scored taxa with only a few unisexual members as bisexual; *Uvariopsis*, which is mostly unisexual but includes one bisexual species (*U. bisexualis* VERDCOURT 1986), as uncertain. We previously scored *Ephedranthus* as bisexual, but according to FRIES (1959) and VAN HEUSDEN (1992) it is androdioecious. Our results indicate that departures from bisexual flowers are separate advances in all taxa where they occur.

Calyx

27. Sepals (0) distinct or basally fused, (1) calyptrate.

28. Sepal estivation (in bud) (0) imbricate, (1) valvate.

29. Sepal size (0) small, (1) large, enclosing petals until nearly open.

30. Sepal margins (0) normal, (1) winged.

We combine basally fused and free sepals, which intergrade, and distinguish them from calyptrate (more fused, tearing on anthesis). Based on VAN HEUSDEN (1992), we rescored *Fusaea* as calyptrate. The calyptrate calyx of *Eupomatia* and Himantandraceae is not homologous with the

same condition in Annonaceae, where it is derived in *Fusaea* and *Letestudoxa* (either a parallelism or a synapomorphy lost in *Duguetia* and *Pachypodanthium*) and some *Uvaria* species.

VAN HEUSDEN (1992) reported that many taxa described as having valvate sepals are actually imbricate (*Anaxagorea*, *Cleistopholis*, *Ambavia*, *Greenwayodendron*), whereas *Sapranthus* is valvate. We have confirmed this with our own observations and rescored the taxa accordingly. We rescored *Uvariopsis* as unknown, since the sepals do not meet (called apert by VAN HEUSDEN 1992) and the state must be considered undefined. Our results indicate that imbricate sepals are a synapomorphy of Magnoliaceae and Annonaceae when the two taxa are the sister groups (Fig. 8), but with other arrangements this homology is equivocal, since other outgroups are valvate. In any case, imbricate is basic in Annonaceae and shifts to valvate below *Polyalthia stuhlmannii*. Valvate reverses to imbricate in the *Ephedranthus-Malmea-Pseudoxandra* clade, the same point where petals become imbricate. However, the two characters are not redundant, since they are not correlated elsewhere (e.g., *Guatteria*).

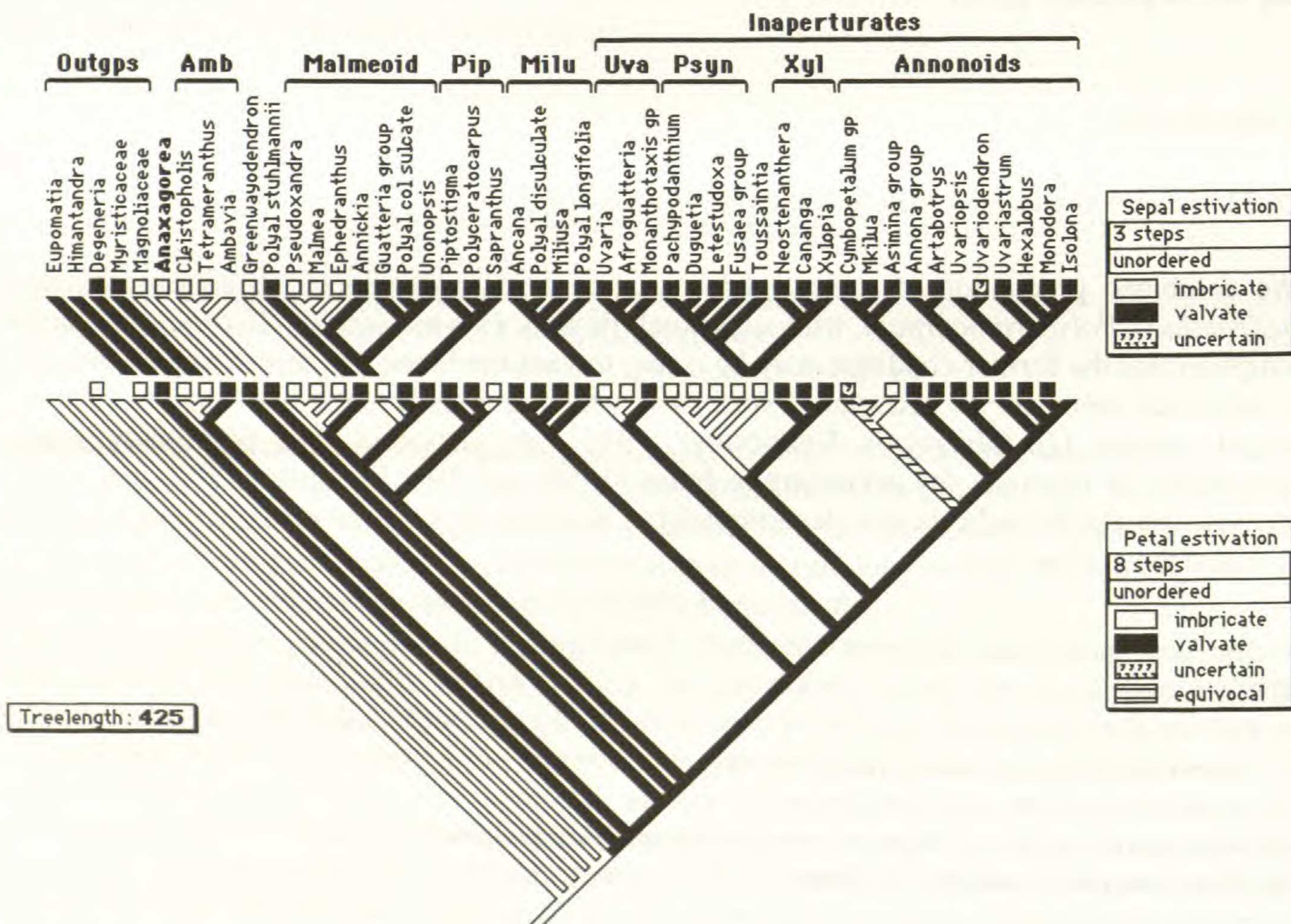


Fig. 8. — Representative most parsimonious tree (island B), showing evolution of sepal (above) and petal estimation.

Large sepals are a conspicuous synapomorphy of *Toussaintia* and the pseudosyncarps. They also originate in *Uvariastrum* and *Hexalobus*; they are a synapomorphy on trees in which these two genera are linked, but on others this is equivocal. In contrast, winged sepal margins (called reduplicate-valvate by VAN HEUSDEN 1992) arise independently everywhere they occur (*Toussaintia*, *Hexalobus*, some members of *Uvaria* and the *Cymbopetalum* group).

Corolla

31. Corolla (0) >2 cycles, (1) 1-2 cycles, (2) absent (or intergrading with androecium in Himantandraceae: ENDRESS 1977) (ordered).

32. Petal estivation (in bud; refers to outer whorl if the two whorls differ) (0) imbricate, (1) valvate.

33. Petal fusion (0) free, (1) fused at least basally.

34. Outer petals (0) subequal, (1) reduced or absent.

35. Inner petals (0) subequal, (1) reduced or absent.

36. Petal shape (0) oval, (1) elongate, pointed (near a length:width ratio of 3:1, petals were scored 0 if oblanceolate, 1 if lanceolate).

37. Petals at anthesis (0) spreading, (1) curved over other floral parts for their whole length, (2) with concave basal portion, often connivent. MORAWETZ (1988), VAN HEUSDEN (1992), and our own observations of herbarium material, photographs, and descriptions in the general references.

Presence of one or two cycles of petals, the basic and nearly universal condition in Annonaceae, is homologous with the same state in Magnoliaceae (e.g., *Liriodendron*, *Magnolia* spp.) when Magnoliaceae or Magnoliaceae plus *Degeneria* are the sister group of Annonaceae, equivocally so otherwise. The more numerous petals of *Toussaintia hallei* (but not *T. congolensis* and *T. orientalis*), suggested to be primitive by SCHATZ & LE THOMAS (1993), are apparently derived within this genus.

For petal estivation, we have rescored *Cleistopholis* as imbricate, based on VAN HEUSDEN (1992) and our own observations; the *Cymbopetalum* group as uncertain rather than valvate, because the basal state is uncertain in terms of the internal cladogram of JOHNSON & MURRAY (1995); and *Isolona* as unknown, because the petals are apert (VAN HEUSDEN 1992). VAN HEUSDEN listed *Mkilua* as imbricate, but VERDCOURT (1971) described the inner whorl as apically slightly imbricate, the outer as valvate; in view of this confused and marginal situation, we have scored *Mkilua* as unknown.

Although other Magnoliales with petals (Magnoliaceae, *Degeneria*) have imbricate estivation, all our analyses imply that valvate petals are basic in Annonaceae, being one of the synapomorphies that arose at the very base of the family (Fig. 8). FRIES (1959) used petal estivation to sort most genera into Uvarieae and Unoneae, but our results confirm the common suspicion (e.g., WALKER 1971a; LE THOMAS 1980/81) that this character is of less general value. However, reversals to imbricate do unite several clades: *Cleistopholis* and *Tetrameranthus*, *Uvaria* and *Afroguatteria*, and *Toussaintia* and the pseudosyncarps (a change from our previous analyses, where imbricate petals were a synapomorphy of the uvarioid clade in its wider sense, and valvate petals of *Monanthotaxis* were derived). In the malmeoids, imbricate petals arise once or twice, depending on the position of *Annickia* (which is valvate): they may be a synapomorphy of *Ephedranthus*, *Malmea*, and *Pseudoxandra*, or equivocally homologous with *Guatteria*. Imbricate

petals are autapomorphic in *Sapranthus*; in *Asimina* they are autapomorphic in island A, equivocally homologous with the *Cymbopetalum* group in island B.

Fusion of the petals (in which we include basal fusion in *Monodora*) is a unique synapomorphy of *Hexalobus* and the parasyncarpous genera *Monodora* and *Isolona*. In contrast, reduction or loss of either the outer (*Annickia*, *Piptostigma*, *Miliusa*) or inner whorl (*Cleistopholis*, *Neostenanthera*, *Monodora*) occurs independently in all taxa where it is seen.

Petal shape poses problems of definition like those in other continuous characters. However, most taxa vary above or below a length:width limit of about 3:1. In the few taxa that vary around this limit (e.g., *Cleistopholis*, *Annickia*, *Toussaintia*, *Artabotrys*), we used shape as a secondary criterion, associating groups with a rounded apex (oblanceolate) with oval, those with a pointed apex (lanceolate) with elongate, since most longer petals are pointed. When the two whorls of petals differ in length (e.g., *Cleistopholis*, *Piptostigma*), we based the scoring on the longer whorl. We scored sympetalous taxa as unknown, since their petal shape is too modified by fusion. Our results indicate that oval petals are primitive; elongate petals are a conspicuous synapomorphy of the xylopioids and at least part of the miliusoids (depending on internal relationships), but they originate independently elsewhere (*Cleistopholis*, *Greenwayodendron*, *Piptostigma*, *Artabotrys*).

In earlier analyses we included a character for floral type, emphasized by MORAWETZ (1988), which is of great interest for pollination biology (cf. GOTTSBERGER 1989). We simplified the three types (A, B, C) of MORAWETZ, which correspond to progressively greater connivence of the petals at anthesis, to two: petals spreading or connivent. We assigned his type B to the spreading state, except for taxa with petals that separate to some degree but are shaped like those of the connivent type (*Cananga*: DEROIN 1988; *Cleistopholis*, *Artabotrys*). Several considerations led us to doubt the validity of this character: the difficulties just mentioned in placing taxa, doubts on scoring without observations on living material, the fact that the connivent state combines types with very different petal form (some arched for their whole length over the other floral parts, others with a pronounced basal concavity), and its high level of homoplasy on the resulting trees.

Because of these problems, we have replaced floral type with a new character that expresses many of the same distinctions but is defined in terms of more easily applied criteria of petal morphology and orientation, rather than function. It might be suspected that this character would be correlated with petal shape, but in fact all combinations of states in the two characters are found. Despite these improvements, the new character is also highly homoplastic. Although *Anaxagorea* has basally concave petals, the basic condition in Annonaceae appears to be spreading petals, as in the outgroups. Basally concave petals arise independently in *Anaxagorea*, *Annickia*, and *Piptostigma*, but they are a synapomorphy of the xylopioids and (in island B) of *Artabotrys* and *Annona*. Curved petals originate independently in *Unonopsis*, *Pachypodanthium*, the *Cymbopetalum* group, and *Monodora*; they may be homologous in the ambavioids and in *Ephedranthus*, *Malmea*, and *Pseudoxandra*, but this is equivocal, since *Tetrameranthus* and *Malmea* have spreading petals.

Stamens

38. Outer staminodes (0) absent, (1) present.

39. Inner staminodes (0) absent, (1) present.

40. Stamen morphology (0) laminar, (1) narrower but with tongue-like extension of connective, (2) peltate-truncate (cap concealing anthers), (3) peltate-apiculate (ordered) (ENDRESS & HUFFORD 1989 for outgroups).

41. Anthers (0) normal, (1) locellate (at maturity).

With three of the five outgroup arrangements, inner staminodes are a primitive feature of *Anaxagorea*, shared with most Magnoliales except Magnoliaceae and Myristicaceae (where we scored the character as unknown, since the flowers are unisexual), which reappear in *Xylopia*. However, their status in *Anaxagorea* is equivocal when Magnoliaceae or Magnoliaceae plus Myristicaceae are the sister group of Annonaceae.

Because VAN HEUSDEN (1992) reported outer staminodes in Asian species of *Uvaria*, we rescored the genus as uncertain for this character. Outer staminodes are less significant than inner; they arise independently in *Xylopia*, *Fusaea*, and *Uvaria*.

In separating tongue-like and peltate-truncate stamen morphology, we emphasized whether the connective cap covers the anthers. *Tetrameranthus umbellatus* and *T. laomae* differ from other species in having a flat rather than tongue-like connective extension (WESTRA 1985), but because this appears to be more cylindrical than cap-like, we scored the genus as a whole as tongue-like. Since *Xylopia* varies between truncate and apiculate (LE THOMAS 1969; VAN HEUSDEN 1992), we have

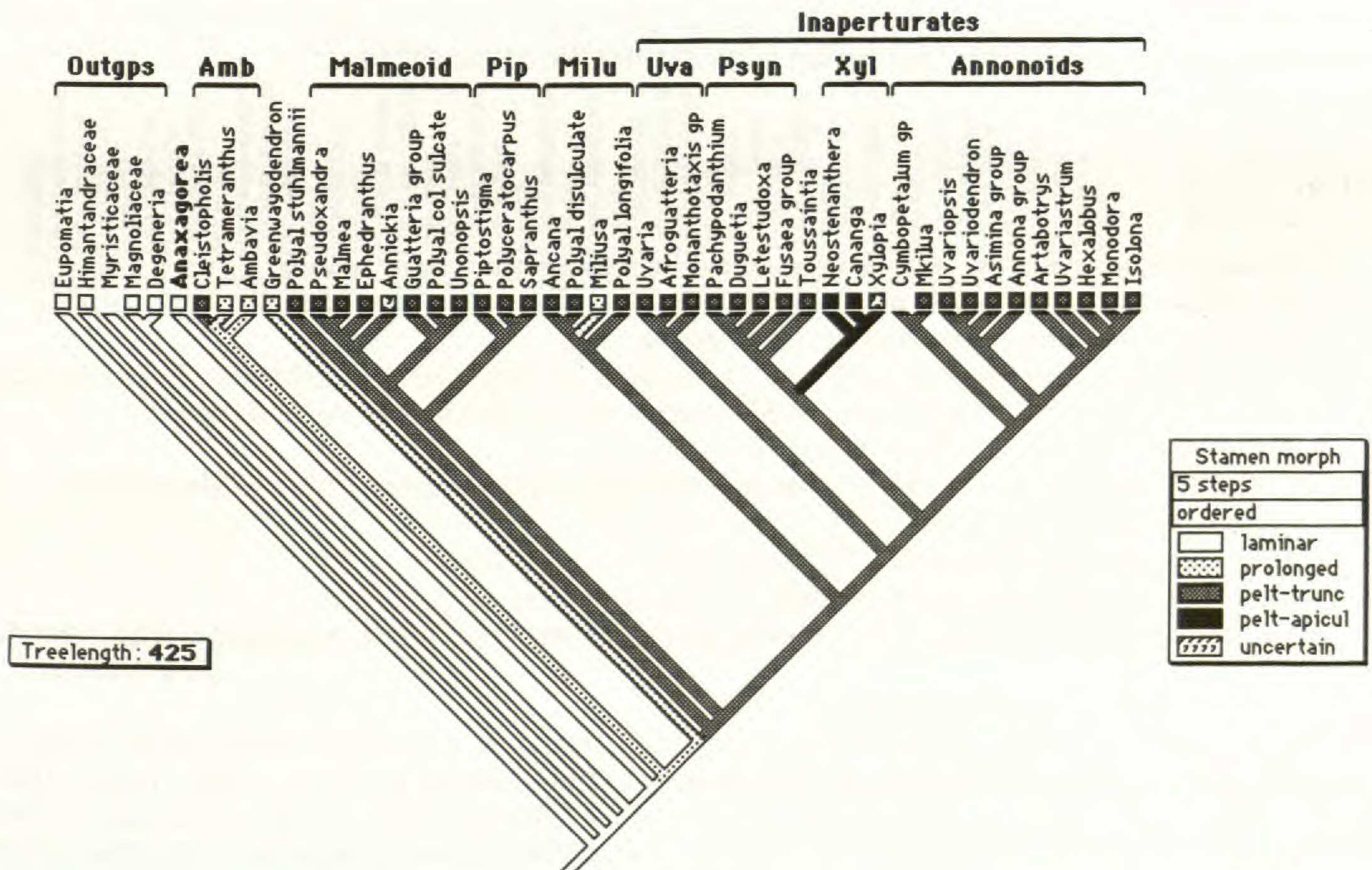


Fig. 9. — Representative most parsimonious tree (island A), showing evolution of stamen morphology.

rescored it as uncertain. Several taxa scored as peltate converge with tongue-like, but those we have examined appear to involve modification of a peltate cap (e.g., *Ancana*, illustrated by MORAWETZ 1988). We previously scored *Uvariopsis* as unknown, since species in Gabon (LE THOMAS 1969) lack a connective extension, but VAN HEUSDEN (1992) described some species as having a discoid cap, so we have rescored the genus as peltate-truncate. We previously scored the *Cymbopetalum* group as peltate-truncate, but because JOHNSON & MURRAY (1995) described the basal genera *Trigynaea* and *Froesiodendron* as having a conical extension and no extension, respectively, and *Cymbopetalum* and *Cardiopetalum* as discoid, we have rescored the group as unknown.

As emphasized previously (DOYLE & LE THOMAS 1994, 1995), stamen morphology is one of the more informative characters (Fig. 9). It contributes to the basal position of *Anaxagorea*, which is unique in having laminar stamens like those of other Magnoliales (cf. WALKER 1971a). Stamens become narrower but retain a tongue-like extension in the ambavioids and *Greenwayodendron*, then develop the peltate connective characteristic of most of the family. Origin of an apiculus on the peltate connective unites the xylopioids. The only cases of homoplasy are convergent origin of a peltate connective in *Cleistopholis* and reversal to a tongue-like connective in *Miliusa* (also interpreted as secondary by WALKER 1971a).

Locellate anthers occur in two unrelated groups, both with compound pollen: the xylopioids, and *Mkilua* and the *Cymbopetalum* group. This is equivocal as a synapomorphy of the xylopioids, since anthers are locellate in *Neostenanthera* and *Xylopia* but not in *Cananga*. Interestingly, *Cananga* does have septations in its early stamen ontogeny; however, this is also true of at least some other groups with compound pollen (DAVIS 1966; WALKER 1971a).

Pollen

42. Pollen unit (0) single (monads), (1) compound (tetrads, polyads).
43. Proximal exine (0) normal, (1) reduced.
44. Aperture (0) sulcate, (1) inaperturate, (2) sulculate.
45. Pollen size (average) (0) small (<45 μm), (1) medium, (2) large (>90 μm) (ordered). Based on acetylated material studied with LM rather than SEM material, which often appears to be shrunken.
46. Pollen shape (0) elongate (boat-shaped), (1) globose.
47. Tectum (0) imperforate (or with very small perforations), (1) verrucate, (2) reticulate-perforate.
48. Spinules (0) absent, (1) present.
49. Infratectal structure (0) granular, (1) intermediate, (2) columellar.
50. Base of infratectum (0) undifferentiated, (1) fused granules.
51. Nexine foliations (0) absent, (1) 1-2, often discontinuous, (2) multiple, continuous, often contorted (ordered).
52. Outer foliation (0) undifferentiated, (1) thickened.

General: WALKER (1971a, 1971b, 1972b, 1976), LE THOMAS & LUGARDON (1974, 1975, 1976), LE THOMAS (1980/81, 1988), LE THOMAS & THANIKAIMONI (1987); HESSE & WAHA (1984), WAHA (1985), MORAWETZ & WAHA (1985), LE THOMAS et al. (1986), WAHA & HESSE (1988), WAHA & MORAWETZ (1988), ROGSTAD & LE THOMAS (1989); *Fusaea*: LE THOMAS et al. (1994); *Greenwayodendron*, *Polyalthia* groups: our observations of pollen preparations, unpublished photos of THANIKAIMONI; Himantandraceae: PRAKASH et al. (1984), LUGARDON (pers. comm.); Magnoliaceae: PRAGLOWSKI (1974); Myristicaceae: WALKER & WALKER (1981).

We scored exine structure characters (47, 49-52) as unknown in taxa with reduced exines, except in two cases where the layer in question is well enough developed to be categorized (infratectum in *Monanthotaxis*, tectum in *Duguetia*). Exine ultrastructure in *Afroguatteria* is based on LE THOMAS (1980/81, overlooked in construction of the matrix in DOYLE & LE THOMAS 1995). We previously hesitated to score ultrastructure in *Sapranthus*, because its exine appeared to be too reduced in the low-magnification figures of WAHA & HESSE (1988), but closer examination indicates that it can be scored as having a granular infratectum, no fusion of basal granules, and no foliations.

Pollen characters have been treated in such detail in our previous articles (DOYLE & LE THOMAS 1994, 1995; LE THOMAS et al. 1994) that we concentrate here on the most important agreements with and changes from our earlier analyses. In Fig. 10, sketches of the principal pollen types are superimposed on a tree showing evolution of the infratectal structure.

As in our previous analyses, our results indicate that the basic pollen type in Annonaceae was like that of *Anaxagorea* (Fig. 11A, B): single, boat-shaped, monosulcate pollen with a continuous tectum and granular infratectal structure, as in most other Magnoliales (except for the intermediate or columellar structure of Magnoliaceae and many Myristicaceae). Presence of 1-2 nexine folia-

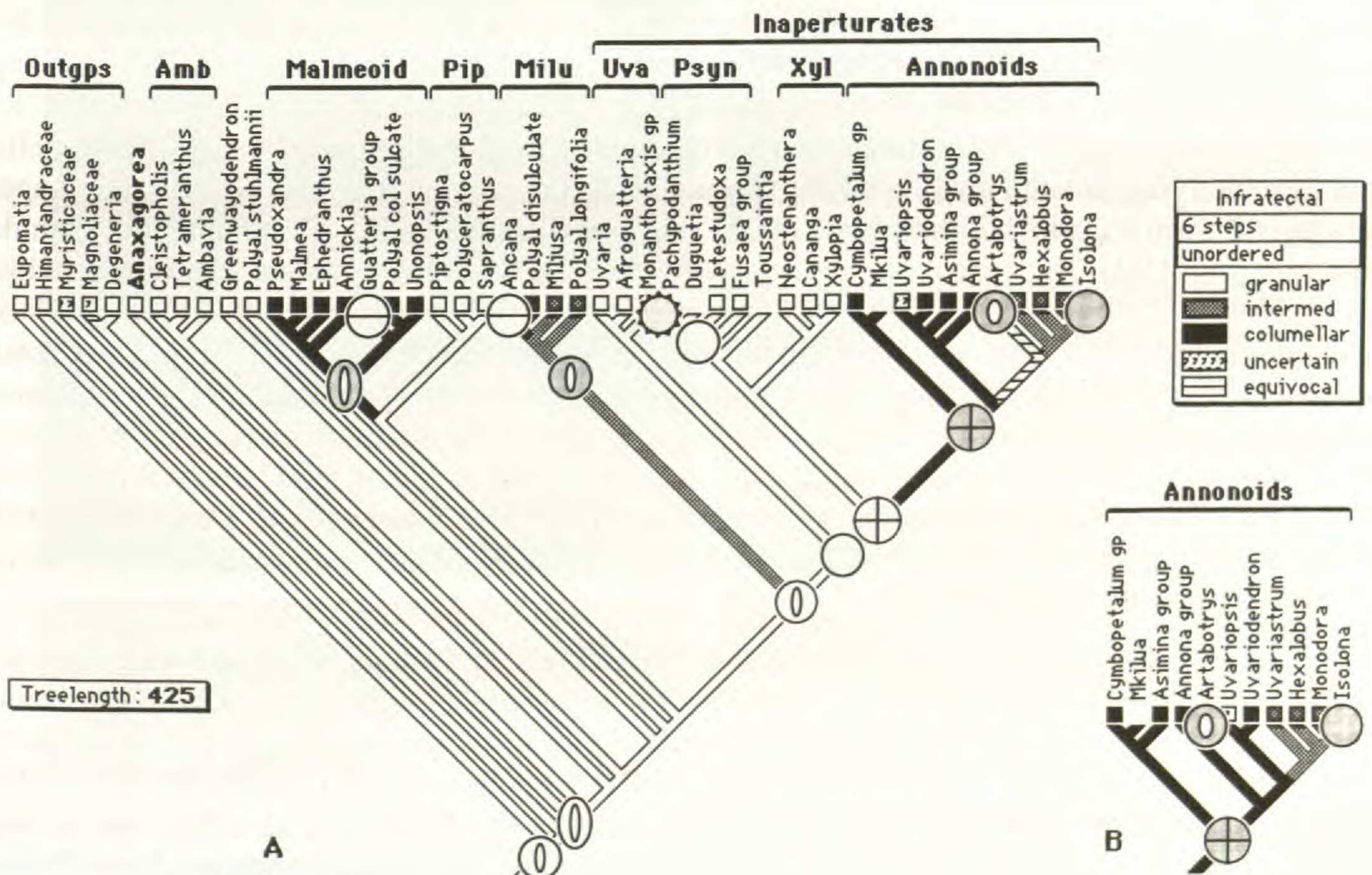


Fig. 10. — Representative most parsimonious trees, with sketches of major pollen types placed at the point where they originate on a cladogram showing evolution of infratectal structure: **A**, island A; **B**, island B.

tions is basic (also seen in Magnoliaceae and Myristicaceae and homologous with one or both taxa on all trees: Fig. 12). The lack of nexine foliations in *Piptostigma* and *Sapranthus* is not primitive, as suggested for *Piptostigma* by LE THOMAS (1980/81), but a reversal. The reticulate-columellar monosulcate pollen of the malmeoids (Fig. 11C, D) is derived directly from the *Anaxagorea* type. In some trees of DOYLE & LE THOMAS (1995), the granular monosulcate pollen of *Piptostigma* and *Polyceratocarpus* was secondarily derived from the malmeoid type, but in the present analysis it is primitive. A shift to globose pollen shape is a synapomorphy of the miliusoids and inaperturates, but it occurs independently in *Tetrameranthus* and *Sapranthus*. This implies that boat-shaped pollen is primitive everywhere it occurs in Annonaceae. In contrast, in DOYLE & LE THOMAS (1994) the shift to globose pollen occurred lower and reversed to boat-shaped in the piplostigmoid-malmeoid clade, while in DOYLE & LE THOMAS (1995) globose shape originated independently in the miliusoids and the inaperturates.

It is in the inaperturates that scenarios for pollen evolution differ most from our previous analyses. In DOYLE & LE THOMAS (1994; Fig. 1A), the inaperturates formed two lines nearer the base of the tree, of which the *Artabotrys*-annonoid line became columellar (Fig. 11E, F). In DOYLE & LE THOMAS (1995; Fig. 1B), these two lines formed a single clade, with the columellar annonoids at the base, and linked with columellar monosulcates by *Annickia* and *Artabotrys*. Thus both the granular tetrads of the xylopioids and the granular single grains of most uvarioids sensu lato were derived from columellar tetrads. Since the inferred reversal from columellae to granules is correlated with a thinner exine, we suggested it might be a developmental consequence of exine reduction, recalling the views of WAHA (1988) on origin of "fragile" exines in Annonaceae. We also suggested that the thick basal layer of the xylopioids (Fig. 11G), interpreted by LE THOMAS (1980/81) as derived from fused granules, might instead be derived from the expanded bases of columellae and an underlying foliated layer, as in the annonoids. In the present analysis (Fig. 10), the inaperturates still form a clade, but their basic pollen type, seen in the uvarioids, is single with granular structure (Fig. 11H). This type is derived relative to the miliusoids by loss of the aperture (a unique event) and multiplication of nexine foliations (which occurs elsewhere only in *Tetrameranthus*). Columellae and a reticulate tectum originate later, in the annonoids (Fig. 11E, F), independently from those of the malmeoids; this restores annonoid pollen to its previous status as one of the most advanced types in Annonaceae. Although the single grains of the uvarioids are primitive, those of the pseudosyncarps are derived from tetrads; *Fusaea* retains granular tetrads homologous with those of the xylopioids (recalling WALKER 1971a, contrary to LE THOMAS et al. 1994). *Toussaintia* differs in having an extremely reduced exine, but its tetrads of globose grains and foliated nexine are consistent with its position.

In DOYLE & LE THOMAS (1994), we observed that tetrads are usually correlated with reduction of the proximal exine (cf. LE THOMAS 1980/81), but with three exceptions: *Pseudoxandra*, which has tetrads but a normal proximal exine; and *Ambavia* and *Isolona*, which have a reduced proximal exine but single grains. Since *Isolona* was nested among the annonoid tetrads, we inferred that its reduced proximal exine is evidence of a tetrad ancestry (LE THOMAS et al. 1986), but no such argument can be made for *Ambavia*. In the present analysis, the pseudosyncarps above *Fusaea* have monads derived from tetrads, but they show no proximal reduction. This implies that secondary monads may or may not retain a reduced proximal exine. The single grains of *Artabotrys* pose a special problem. In our previous analyses, where *Artabotrys* formed a link between monosulca-

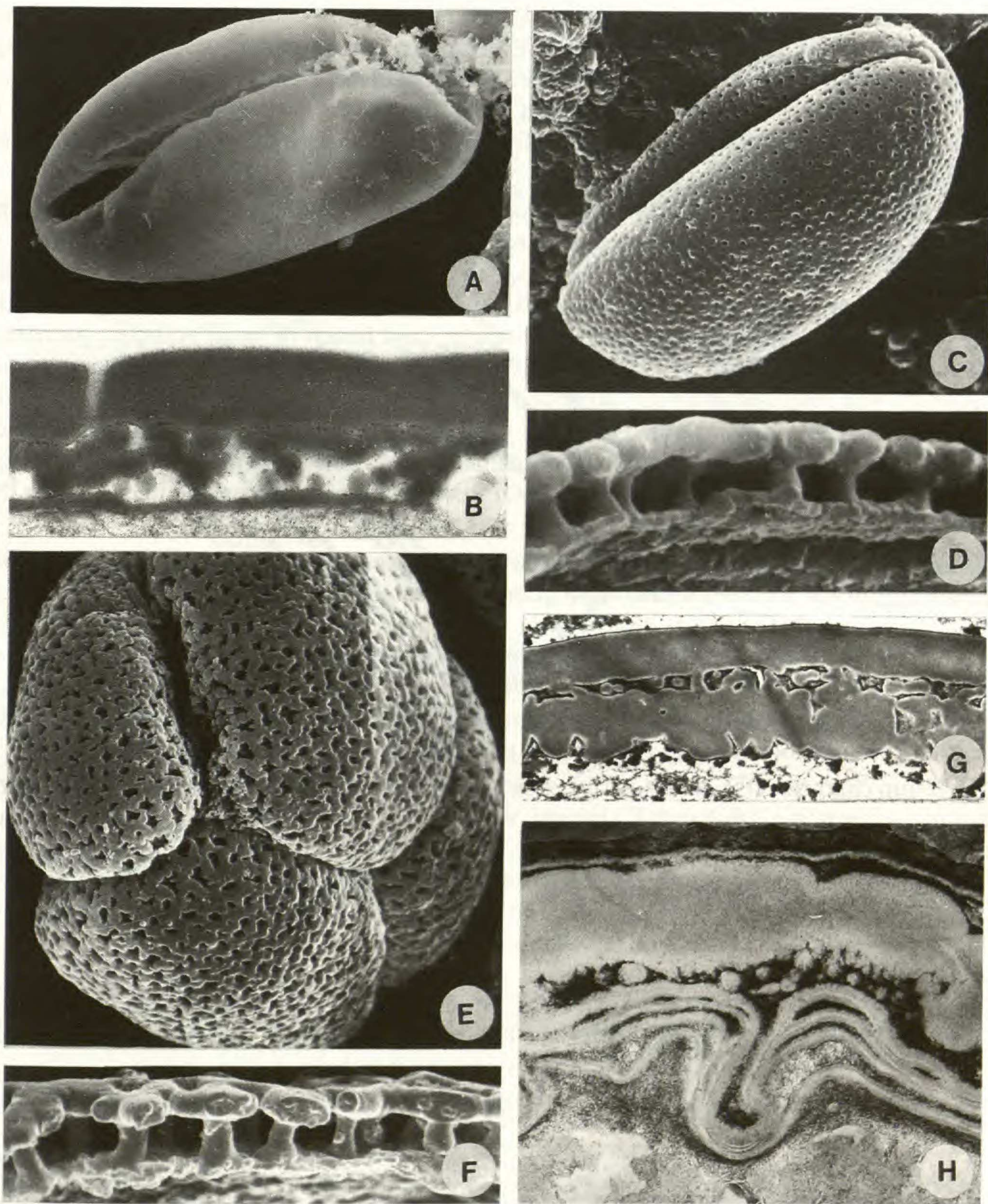


Fig. 11. — Pollen types and exine structures in Annonaceae (SEM and TEM). — *Anaxagorea dolichocarpa* Sprague & Sandwith: **A**, monosulcate pollen, $\times 1200$. — *Anaxagorea brevipes* Benth.: **B**, granular infratectal structure, few nexine foliations, $\times 27,000$. — *Polyalthia capuronii* Cav. & Ker.: **C**, monosulcate pollen, $\times 1300$. — *Polyalthia heteropetala* Diels: **D**, columellar infratectal structure, $\times 800$. — *Annona glauca* Schum. & Thonn.: **E**, inaperturate tetrad, $\times 675$; **F**, columellar infratectal structure, $\times 3400$. — *Xylopia staudtii* Engl. & Diels: **G**, granular infratectal structure, nexine of fused granules, $\times 18,000$. — *Uvaria klaineana* Engl. & Diels: **H**, granular infratectal structure, nexine of multiple foliations, $\times 30,000$.

te monads and inaperturate tetrads, its poorly developed aperture could be interpreted as a vestigial sulcus, but in our present analysis it is a reversal. This suggests the possibility that the aperture is not distal but rather a vestige of a reduced proximal exine, as in *Isolona*. The intine is reduced at the aperture (LE THOMAS 1988), which is not true of the proximal intine in *Isolona* (LE THOMAS et al. 1986); however, observations of young grains in tetrads are needed for a definitive answer to this problem.

Since the granular structure of the uvarioids and xylopioids now appears to be primitive, there is no need to explain it as a consequence of reduction. However, the nexine of xylopioids still poses problems: it shows no trace of foliations, and instead appears to consist of enlarged, fused granules (Fig. 11G). On our trees, origin of this structure involves both loss of multiple foliations (a two-step change, since this is an ordered character) and fusion of basal granules. As noted in DOYLE & LE THOMAS (1994, 1995), this fusion is unrelated to the less complete fusion in *Cleistopholis* and *Polyceratocarpus*, suggested as a transitional stage by LE THOMAS (1980/81), since these genera are well separated from the xylopioids (and each other). While this layer cannot be explained as derived from bases of columellae, it may be worthwhile to consider the possibility that foliations are involved. Developmental studies might be informative in evaluating these possibilities.

LE THOMAS (1980/81) hypothesized that the intermediate infratectal structure of some annonoids and miliusoids was transitional from granular to columellar. In the annonoids, the direction of evolution in this character was equivocal in DOYLE & LE THOMAS (1994), but in DOYLE & LE THOMAS (1995) and the present analysis the intermediate state is derived from columellar and a synapomorphy of *Uvariastrum*, *Hexalobus*, and the parasyncarps on most trees. However, in the miliusoids, there is indeed a succession from granular in the outgroups to intermediate at the base of the group, and to columellar in *Ancana* and the disulculate *Polyalthia* group.

The tectum character (Fig. 12) is partly correlated with infratectal structure, but with important exceptions. A verrucate ("dislocated") tectum, which LE THOMAS (1980/81) suggested was transitional between the continuous and reticulate types, is a synapomorphy of the miliusoids and of *Letestudoxa* and *Duguetia*, in both cases derived from continuous but unrelated to reticulate, whereas in *Isolona* it is derived from reticulate. The reticulate tectum of the malmeoids and annonoids originates directly from a continuous tectum and coincides with the origin of columellae, but in the miliusoids the tectum remains verrucate even when the infratectum becomes columellar (*Ancana* and the disulculate *Polyalthia* group). Spines originate independently in *Monanthotaxis* and *Pachypodanthium*, consistent with their different size and form in the two taxa (DOYLE & LE THOMAS 1995). The spines of *Pachypodanthium* are probably derived from verrucae, as seen in the related genera *Duguetia* and *Letestudoxa*, but outgroups do not reveal any precursors for the spines of *Monanthotaxis*.

Thickening of the outer nexine foliation is the most homoplastic pollen character. It is often correlated with columellar infrastructure, but not entirely. Besides arising independently in Magnoliaceae and Myristicaceae, it is a synapomorphy of the malmeoids, of *Ancana* and the disulculate *Polyalthia* group, and of part or all of the annonoids, which is reversed in *Hexalobus* and *Isolona* but not in *Monodora*.

Origin of the di- or zonosulculate aperture condition is a synapomorphy of the miliusoids other than *Polyalthia longifolia* (which has a reduced sulcus), but it occurs independently in *Sapranthus*,

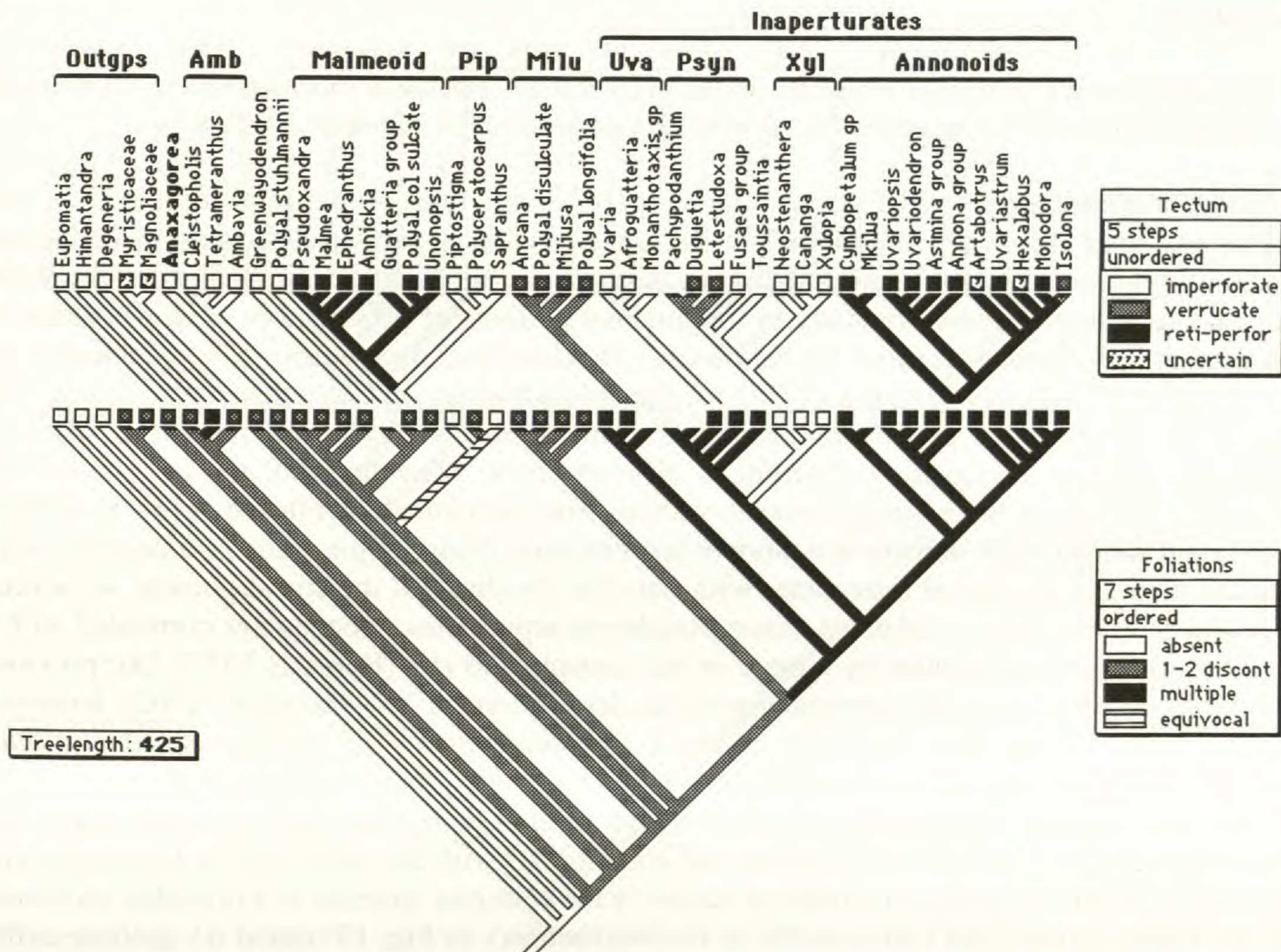


Fig. 12. — Representative most parsimonious tree (island A), showing evolution of the tectum (above) and nexine foliations.

Guatteria, *Afroguatteria*, and *Letestudoxa*. WALKER (1971a) proposed that disulculate pollen was derived from inaperturate; our results support this for *Afroguatteria* and *Letestudoxa*, but they imply that *Sapranthus*, *Guatteria*, and disulculate miliusoids were derived directly from monosulcate ancestors.

Pollen size is highly homoplastic, but it does show some general trends. Depending on outgroup arrangements, the basic size in Annonaceae is small or equivocal (small or medium), since pollen is medium-sized (45-90 μm , large compared to most angiosperms) in Magnoliaceae, *Degeneria*, and *Anaxagorea*, but small in other Magnoliales, ambavioids, and *Greenwayodendron*. One increase to medium-sized unites the piptostigmoids and malmeoids; another the xylopioids, pseudosyncarps, and annonoids, coinciding with the origin of tetrads; another is an autapomorphy of *Uvaria*. Further size increases unite *Asimina*, *Mkilua*, and the *Cymbopetalum* group in island B and occur independently in *Fusaea*, *Cananga*, and *Annona* (equivocally homologous with *Asimina* in island A). Secondary reduction to small pollen occurs in *Pachypodanthium*.

Receptacle

53. Stamen-bearing portion of receptacle (0) flat or conical, (1) globose or short cylindrical, (2) elongate.
 54. Apex of receptacle (carpel-bearing portion) (0) elongate, (1) flat or convex, (2) concave.

We have treated shape of the stamen- and carpel-bearing zones of the floral receptacle as two characters, since they vary independently. Receptacle shape is hard to determine in herbarium material, and probably for this reason literature descriptions are inconsistent. We defined the states recognized here after extensive study of longitudinal sections of preserved or boiled material of most genera of Annonaceae and Myristicaceae (*Mauloutchia*, *Brochoneura*), supplemented by BAILEY et al. (1943) and ENDRESS (1977) for Himantandraceae, and ENDRESS (pers. comm.) for *Degeneria*. In the lower part of the receptacle, form is correlated with orientation of stamens, which we used as a secondary criterion in difficult cases. Most taxa fall toward one of two extremes: globose, with radiating stamens, varying from horizontal or reflexed below to vertical above; and conical, with stamens at a more or less constant oblique angle. Globose intergrades with cylindrical, whereas conical intergrades with flat. For the shape of the apical portion, we scored pseudosyncarpous taxa as unknown, since pseudosyncarpy seems to be always correlated with a raised central mass surrounded by a more or less conspicuous rim (ENDRESS 1977). Our previous scoring of taxa was generally consistent with the descriptions of VAN HEUSDEN (1992); however, on the basis of her data we have reduced the uncertainties in scoring *Tetrameranthus*, *Ephedranthus*, and *Mkilua*, increased them in *Uvaria* and the *Cymbopetalum* group.

The lower portion of the receptacle shows a high level of homoplasy, but our results imply that flat-conical shape is basic in Annonaceae and homologous with the same state in *Eupomatia* and *Degeneria*. A shift to globose-cylindrical occurs in the common ancestor of *Polyalthia stuhlmannii* and higher groups (and convergently in *Tetrameranthus*). In Fig. 13 (island A), globose-cylindrical reverts to flat-conical in the common ancestor of xylopioids, pseudosyncarps, and annonoids, as well as in *Annickia* and *Ancana*. Within the inaperturates, reversals to globose-cylindrical are synapomorphies of *Duguetia* and *Pachypodanthium* and of part of the annonoids. However, the polarity of the two states is equivocal in island B, where globose-cylindrical annonoids are more dispersed. SCHATZ & LE THOMAS (1993) suggested that the elongate receptacle of *Toussaintia* is primitive and homologous with that of Magnoliaceae, but our results indicate that this feature is derived independently everywhere it occurs (from flat-conical in *Toussaintia* and Magnoliaceae, from globose-cylindrical in *Miliusa* and *Uvariopsis*).

The receptacle apex is somewhat less homoplastic (Fig. 13). It appears to be primitively flat-convex in Annonaceae, rather than concave or elongate in other Magnoliales. A concave apex reappears several times. In some trees, it is a synapomorphy of the malmeoid-piptostigmoid clade, with reversals in *Annickia* and *Pseudoxandra*. It is a synapomorphy of *Xylopia* and *Cananga* in all trees, and of *Asimina* and *Uvariodendron* in island A. An elongate apex is derived independently in *Miliusa*, *Uvariopsis*, and Magnoliaceae. Elongation of the two zones of the receptacle is correlated in the last three taxa, but not in *Toussaintia*.

Carpels

55. Average number of carpels (0) 1 (rarely 2), (1) 2-10, (2) more than 10.

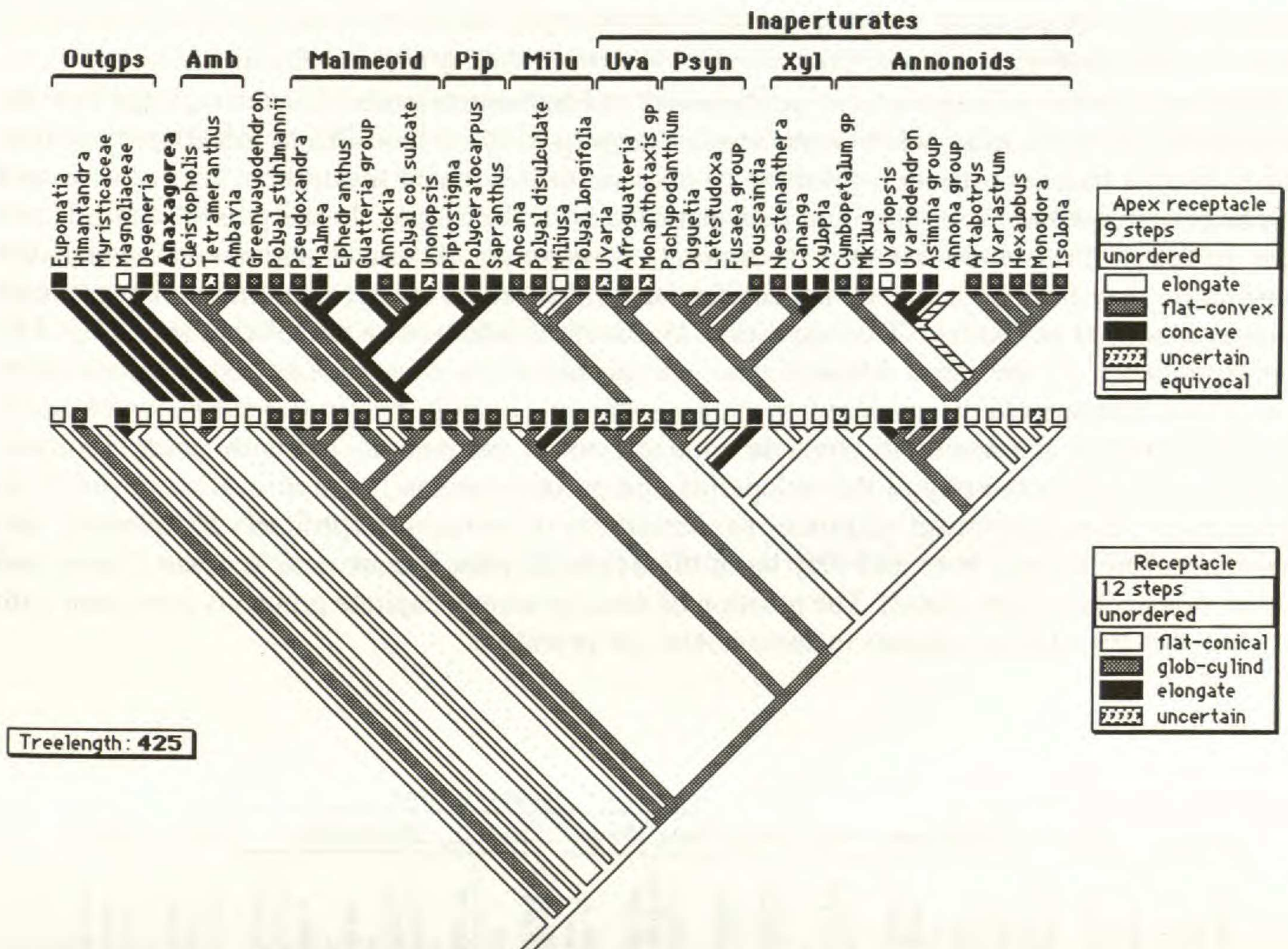


Fig. 13. — Representative most parsimonious tree (island A), showing evolution of the receptacle apex (above) and stamen-bearing portion of the receptacle.

56. Stigma (0) sessile, (1) capitate (base strongly constricted), (2) more or less elongate style.

57. Ovules (0) numerous, (1) two lateral, (2) two basal, (3) one basal.

In DOYLE & LE THOMAS (1994, 1995) we included a character expressing maximum number of monocarps in the fruit stage, for which comprehensive data were presented by VAN SETTEN & KOEK-NOORMAN (1992), but in the present analysis we have replaced this with average carpel number, based on general references (e.g., LE THOMAS 1969). The two characters are correlated, but carpel number seems less likely to be affected by phenotypic plasticity. Compiling numbers for numerous taxa revealed a break at around 11, which led to the limit chosen (the same as between “few” vs. “many” in VAN HEUSDEN 1992). Carpel number may seem preferable theoretically, but it is actually even more homoplastic than monocarp number. The original carpel number in Annonaceae is high, as in the outgroups (except Myristicaceae and *Degeneria*, which have one carpel). Reduction is restricted to a few clades (ambavioids, piptostigmoids, miliusoids, and anno-

noids), but it is almost never an unequivocal synapomorphy because of exceptions within clades, except for the *Artabotrys-Uvariastrum-Hexalobus*-parasyncarp group (island A).

Based on a survey of stigma form in preserved and herbarium material, we concluded that the most consistent distinctions are between sessile (ranging from cushion-like to lobed) and capitate, with a distinct basal constriction (globose or obovate, like a match head; these may abscise and thus be misinterpreted as sessile), and between capitate and elongate. In the present analysis, we have restricted the sessile category somewhat, reassigning obconical stigmas (in which the constriction may not be visible) to the capitate state; this led us to rescore some taxa previously considered sessile as capitate. This appears to be the most informative carpel character (Fig. 14). Sessile stigmas, as in other Magnoliales, are primitive in Annonaceae and retained from *Anaxagorea* through *Greenwayodendron*. Stigmas become capitate below *Polyalthia stuhlmannii*, and this condition is retained through the piptostigmoids, malmeoids, and miliusoids. Elongate stigmas are a synapomorphy of the xylopioids and pseudosyncarps, with a reversal to sessile in *Toussaintia*. Reversal from capitate to sessile is a synapomorphy of *Uvariopsis* and *Uvarioidendron* in some trees and may be of the uvarioids (this is equivocal because *Uvaria* and *Monanthotaxis* have both states). The position of *Annona* among capitate groups is consistent with the view that the capitate stigmas in section *Atta* are primitive.

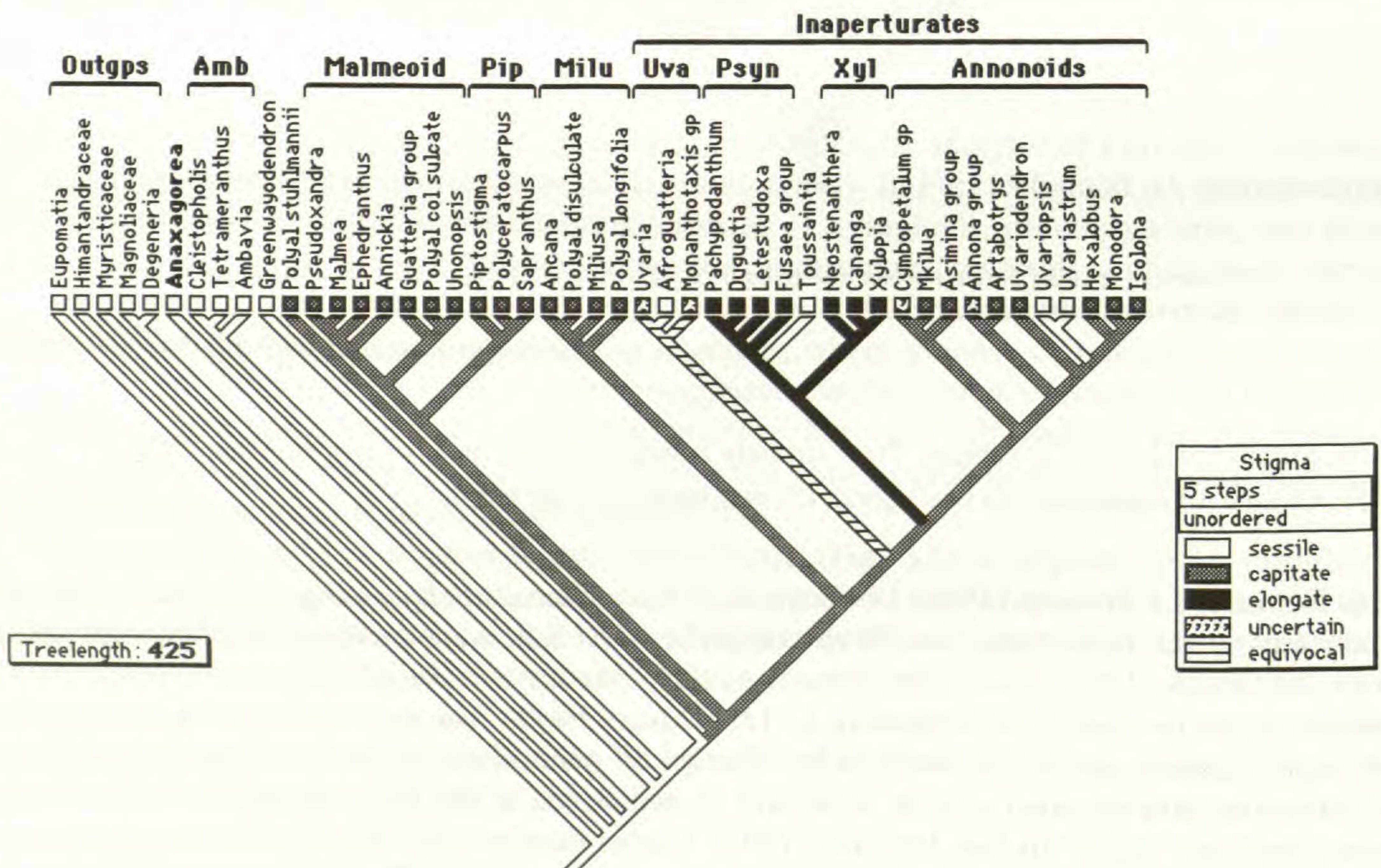


Fig. 14. — Representative most parsimonious tree (island B), showing evolution of stigma form.

Ovule number, a character much discussed in Annonaceae (e.g., WALKER 1971a; LE THOMAS 1980/81), shows more homoplasy and ambiguity, which extend into the outgroups (Fig. 15). With most outgroup arrangements, the reconstructed basal state in Annonaceae is either numerous (as in *Degeneria* and some Magnoliaceae), two lateral (as in other Magnoliaceae, the ambavioids, and *Greenwayodendron*), or two basal (as in *Anaxagorea*); with Myristicaceae as sister group, it may also be one basal. The reconstructed state between *Anaxagorea* and *Polyalthia stuhlmannii* is either numerous or two lateral, but above this it is either numerous or one basal. In either case, the piptostigmoids are basically multiovulate, the malmeoids uniovulate, and there are no reversals in either clade (except within *Unonopsis*). *Pseudoxandra* has one lateral ovule (an autapomorphy scored 0/1/3 to permit several origins); our results imply that this is derived from one basal. The basic state in the miliusoids is still equivocal (they vary between uni- to multiovulate). However, multiovulate is unambiguously basic in the inaperturate clade, with reductions to two basal in *Artabotrys*, and to one basal in *Neostenanthera*, *Annona*, and the pseudosyncarps. Reduction to one ovule is correlated with carpel fusion in both *Annona* and the pseudosyncarps, suggesting a functional and/or developmental correlation (LE THOMAS 1980/81).

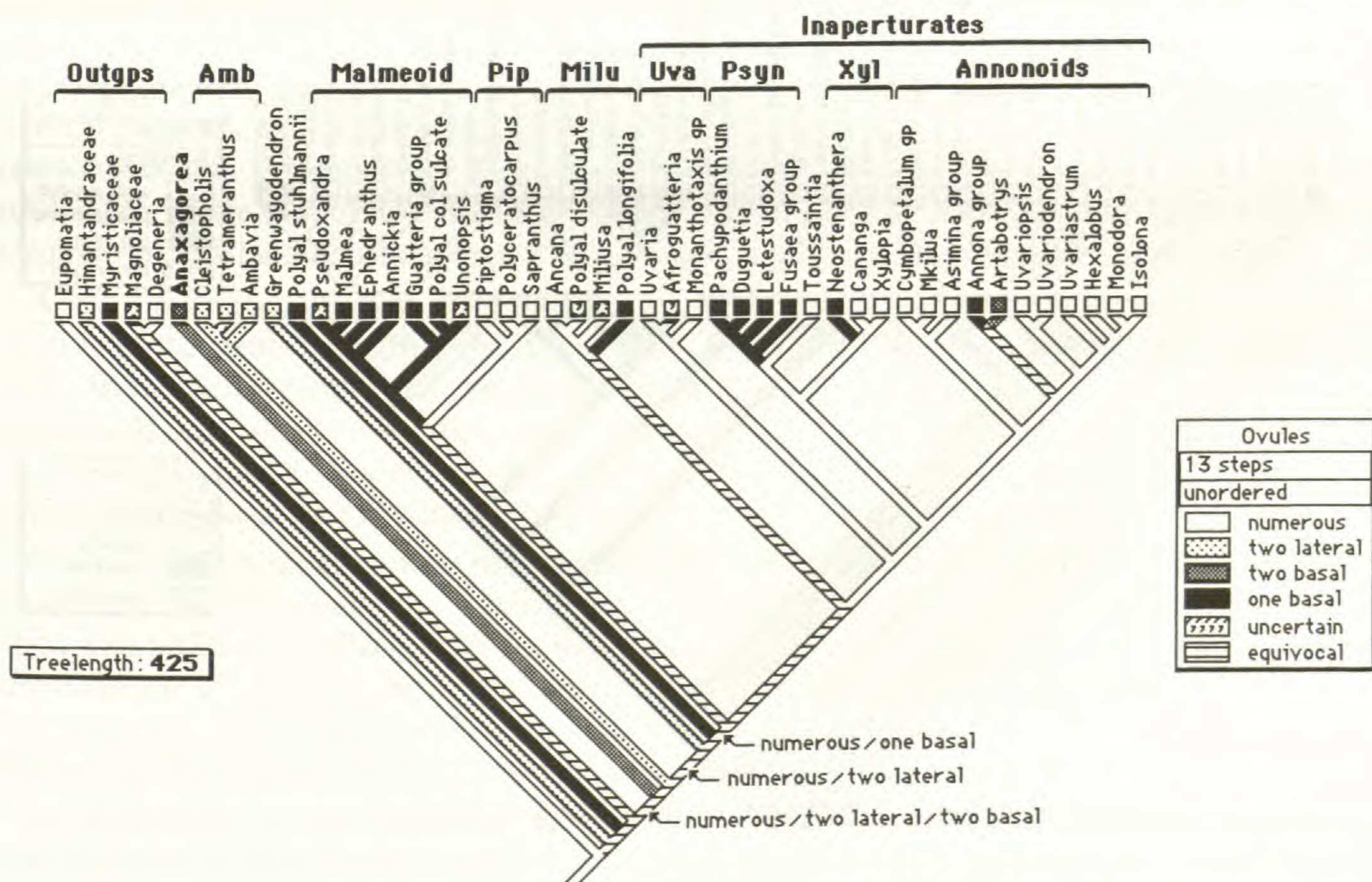


Fig. 15. — Representative most parsimonious tree (island B), showing evolution of ovule number and position, with reconstructed states at important nodes.

Fruits

58. Fruit (0) apocarpous, (1) pseudosyncarpous (at least basally), (2) parasympicarpous.
 59. Basal ring (in pseudosyncarps) (0) absent, (1) formed from receptacle, (2) formed from carpels.
 60. Base of monocarp (0) sessile or nearly so, (1) stipitate.
 61. Articulation of stipe (0) basal, (1) apical.
 62. Fruit (0) ventrally dehiscent, (1) indehiscent, (2) dorsally dehiscent.
 63. Fruit wall (0) thick (>1 mm, generally woody or leathery), (1) thin (<1 mm, generally juicy).

58-63 based on VAN SETTEN & KOEK-NOORMAN (1992), supplemented by our observations for 58-61 and 63.

A highly informative character is carpel fusion (Fig. 16). Pseudosyncarpy, where the carpels are separate or joined only at the base early in ontogeny but become fused as they mature, is an obvious synapomorphy of the pseudosyncarp clade, which independently unites the *Annona* group. It appears that fruits of the pseudosyncarps originally had a basal collar derived from the

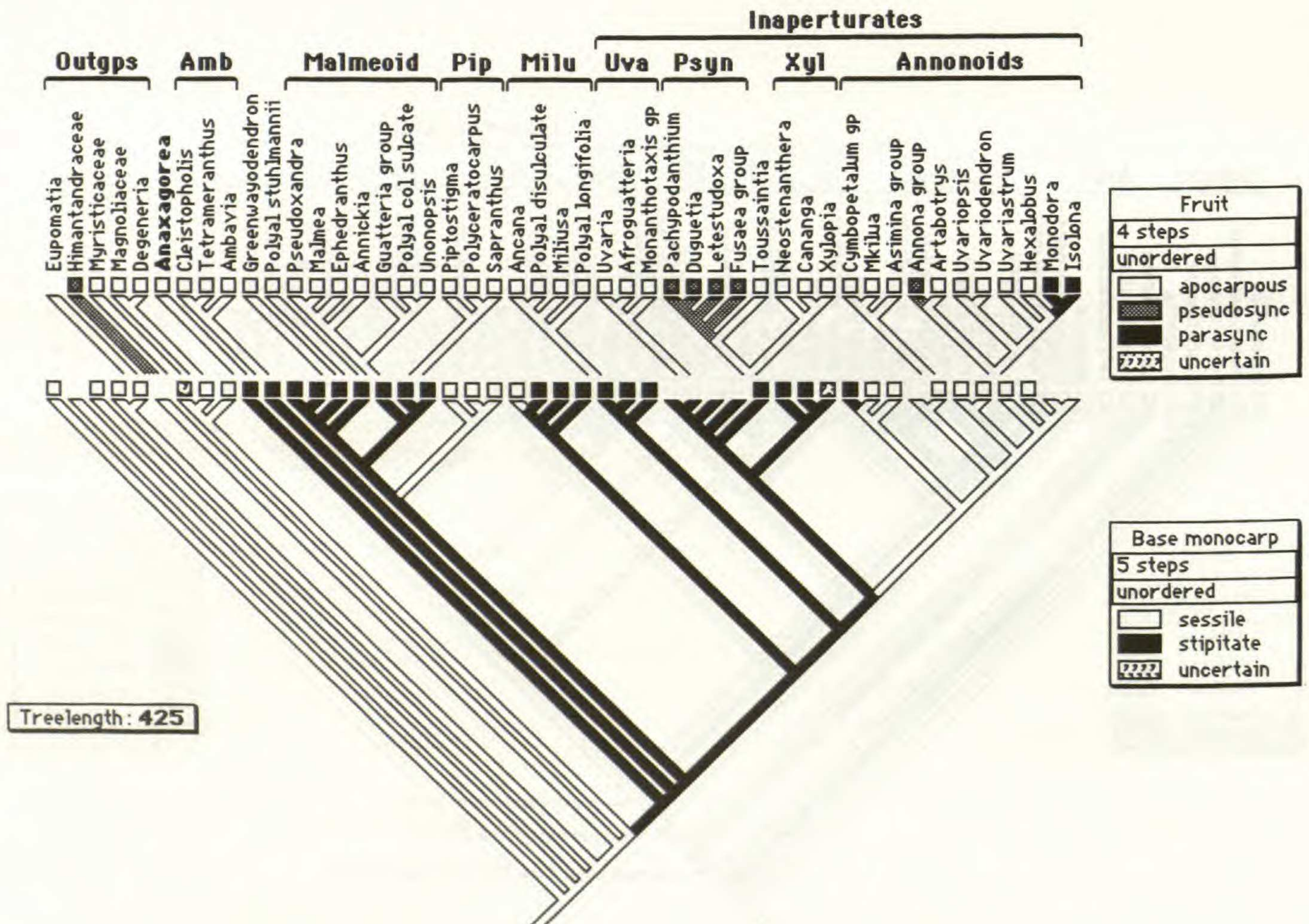


Fig. 16. — Representative most parsimonious tree (island B), showing evolution of fruit type (above) and base of monocarps.

receptacle, as in *Fusaea* and *Letestudoxa*, but this was replaced by a collar derived from sterilized carpels in the common ancestor of *Duguetia* and *Pachypodanthium*. Parasyncarpy, a unilocular gynoecium with parietal placentation, is a separate advance that unites *Monodora* and *Isolona*. Our results do not support the view that the parasyncarpous gynoecium was derived by subdivision of a single carpel (LEINS & ERBAR 1980; ENDRESS 1990). The most closely related taxa, *Hexalobus* and *Uvariastrum*, have several carpels, supporting instead the view that parasyncarpy arose by fusion of several carpels at their margins (DEROIN 1985). ENDRESS stressed the fact that there are Annonaceae with one carpel, but although none of these were included in our analyses, the available data suggest they are not related to the parasyncarps. *Monocarpia* has monosulcate pollen (WALKER 1971a), sessile monocarps, and spiniform endosperm ruminations, consistent with a relationship with *Piptostigma* and *Polyceratocarpus*, as proposed by VAN SETTEN & KOEK-NOORMAN (1992). *Mezzettia* has two lateral ovules per carpel, a third integument (as in *Cleistopholis*), thick ruminations, and the chromosome number $n = 7$ (OKADA & UEDA 1984), suggesting that it belongs near *Cleistopholis* in the ambavioids, as proposed by VAN SETTEN & KOEK-NOORMAN (1992).

Individual monocarps of many Annonaceae are conspicuously stipitate, but others are sessile. We scored taxa with a very short stipe (e.g., *Sapranthus*) as sessile, since the two conditions intergrade. We scored *Anaxagorea* as unknown, since its monocarps taper gradually and the locule extends down into the stipe (DEROIN pers. comm.), unlike typical representatives of either state. We also scored this character and the next as unknown in pseudo- and parasyncarpous taxa, since their carpels are so modified by fusion that comparisons with apocarpous groups would be unwarranted. Our results (Fig. 16) confirm that sessile monocarps, as in the outgroups, are primitive. Origin of stipitate monocarps is a synapomorphy of *Greenwayodendron* and higher groups. A reversal to sessile unites the piptostigmoids (this feature favors a lower position in some one-off trees) and occurs independently in *Ancana*. Another reversal is a synapomorphy of the annonoids in island B, where the stipitate monocarps of the *Cymbopetalum* group are reversed again, but this is equivocal in island A, where monocarps of the *Cymbopetalum* group may be primitive.

A few Annonaceae deviate from the majority condition in having apical rather than basal articulation of the monocarps (this character can be scored only in groups with at least a short stipe). Shifts from the original basal condition to apical occur independently in *Cleistopholis* (other ambavioids are sessile, so their state is undefined), *Annickia*, and the xylopioid genera *Neostenanthera* and *Cananga*, where apical articulation may be either homologous or convergent (articulation is basal in *Xylopia*).

Fruits are indehiscent in most Annonaceae, but ventrally dehiscent in *Anaxagorea*, and dorsally dehiscent in *Mkilua* and the *Cymbopetalum* group (added as a state by DOYLE & LE THOMAS 1995, with addition of *Mkilua*). When Magnoliaceae are the sister group of Annonaceae, the dehiscent follicles of *Anaxagorea* are primitive and homologous with those of Magnoliaceae and Myristicaceae, but under other outgroup arrangements this is equivocal. The dorsal dehiscence of *Mkilua* and the *Cymbopetalum* group is a secondary advance that unites these two groups.

As recognized by VAN SETTEN & KOEK-NOORMAN (1992), there is an apparent break in fruit wall thickness at about 1 mm, which is correlated with a difference between juicy and leathery or woody consistency. The original fruit wall thickness is equivocal, since it is thin (but dry!) in *Anaxagorea* and thick and leathery in the outgroups and ambavioids. However, thin, juicy walls are basic above the ambavioids. Reversal to thick, leathery walls is a synapomorphy of the pipto-

stigmoids, of the pseudosyncarps, of the annonoids above *Mkilua* and *Cymbopetalum* (in island A; possibly the whole group in island B), and possibly of *Uvaria* (which is mixed) and *Afroguatteria*; it occurs independently in *Ancana*.

Seeds

64. Chalaza (0) normal, (1) perichalazal (ENDRESS 1980; KOEK-NOORMAN pers. comm.).

65. Raphe (0) more or less pronounced groove, (1) flat, not manifest, or nearly so, (2) more or less pronounced rib (ordered).

66. Micropylar plug (0) absent or small, (1) large.

67. Endosperm (0) normal, (1) glass-like or stony.

68. Colored oil cells in endosperm (0) absent, (1) present.

69. Seed surface (0) smooth or with low sculpture, (1) tuberculate.

70. Aril (0) absent, (1) rudimentary, (2) bilobed.

Scoring of 65-70 based on VAN SETTEN & KOEK-NOORMAN (1992), supplemented by our observations for 65 and 69. Scoring of 65-68 in *Polyalthia* groups (not distinguished in VAN SETTEN & KOEK-NOORMAN 1992) based on unpublished data provided by KOEK-NOORMAN (pers. comm.; DOYLE & LE THOMAS 1995).

71. Sarcotesta (0) absent, (1) present.

72. Integuments (0) two, (1) three.

73. Idioblasts (oil cells) in seed coat (0) in inner integument, (1) absent, (2) in outer integument, (3) in nucellus.

74. Mesotesta fibers (0) absent, (1) longitudinal, (2) crossed, (3) transverse.

Scoring of 71-74 based on CORNER (1949, 1976) and CHRISTMANN (1987).

75. Ruminations (0) absent, (1) thick, irregular plates or pegs, (2) spiniform, (3) lamelliform (including dissected but thin plates) (CORNER 1949, 1976; MOHANA RAO 1982, 1983; ROGSTAD 1989; ROGSTAD & LE THOMAS 1989; VAN SETTEN & KOEK-NOORMAN 1992; KOEK-NOORMAN pers. comm. for *Polyalthia* groups; and our own observations of fresh and herbarium material).

The perichalazal condition, where the raphe forms a ring all around the seed as a result of differential basal growth, is an important synapomorphy of Annonaceae; the only outgroup in which it occurs is *Austrobaileya*.

Shape of the raphe varies from a pronounced groove to a pronounced rib. Our scoring is a simplification of the detailed characterization of variation in taxa by VAN SETTEN & KOEK-NOORMAN (1992). We combined their flat and not manifest categories; we scored taxa that vary between flat and a shallow rib or groove as flat, between a shallow and distinct rib or groove as the distinct state, but between flat and either distinct state as uncertain (e.g., *Annona* group — flat in *Annona*, raised in *Anonidium*). Our results indicate that the basic condition is flat. A grooved raphe arises only in isolated taxa (*Greenwayodendron*, *Ephedranthus*) or within them. A raised raphe may be a synapomorphy of *Mkilua* and the *Cymbopetalum* group (where the basic state is uncertain); the same is true of *Tetrameranthus* (raised) and *Cleistopholis* (mixed). However, raised is a synapomorphy of *Unonopsis*, the columellar-sulcate *Polyalthia* group, and sometimes *Annickia*, and of at least *Uvariastrum*, *Hexalobus*, and the parasyncarps in the annonoids.

Presence of a micropylar plug poses problems, since taxa often vary between no plug and a small plug, or between small and large. We assigned the first sort of variation to state (0), the second to state (1). We were unable to score outgroups for this character, but the internal relation-

ships imply that absent/small is ancestral. Origin of a large plug is a synapomorphy of two major clades, the ambavioids and the inaperturates. However, it arises independently in *Polyceratocarpus* and *Annickia*. It is secondarily reduced in the pseudosyncarps (except *Letestudoxa*), and *Uvariopsis*, *Asimina*, and the *Cymbopetalum* group in the annonoids, various combinations of which are united by this character.

Consistency of the endosperm ranges from soft to extremely hard and glass-like. We combined glass-like and stony and contrasted them with other states recognized by VAN SETTEN & KOEK-NOORMAN (1992). A change to glass-like is a major synapomorphy of the piptostigmoids and malmeoids, with reversals in *Ephedranthus* and *Sapranthus* (which could support an alternative position near the miliusoids). However, it also occurs independently in *Greenwayodendron* and within the disulculate *Polyalthia* group and *Duguetia*.

Colored oil cells in the endosperm are a synapomorphy of the uvarioids and one or two lines in the annonoids: *Uvariopsis* plus *Uvarioidendron*, and the *Uvariastrum-Hexalobus*-parasyncarp clade (one clade in island B). Independent origins occur in *Ambavia* and *Neostenanthera*.

Seed sculpture varies from smooth to finely grooved or pitted, but we found this too subjective and variable within taxa to be scored consistently; furthermore, the difference between grooved vs. pitted reflects the type of ruminations (lamelliform vs. spiniform) and would be redundant. A more distinctive state is strongly tuberculate sculpture. This was a synapomorphy of the ambavioids and *Greenwayodendron* in some of our previous trees, but the present results indicate that it is a parallel advance or a synapomorphy that was reversed above *Greenwayodendron*.

Arils and similar seed appendages show informative patterns. A rudimentary aril (VAN SETTEN & KOEK-NOORMAN 1992) is a synapomorphy of *Toussaintia* and the pseudosyncarps, with an independent origin in *Uvaria* (homologous in our previous analyses). However, it also occurs in some members of several other groups. A bilobed aril unites *Xylopi*a and *Cananga*; it is also a synapomorphy of *Asimina*, *Mkilua*, and the *Cymbopetalum* group in island B. Neither structure appears to be homologous with the fimbriate aril of Myristicaceae or the sarcotesta of Magnoliaceae and *Degeneria*.

DOYLE & LE THOMAS (1994) cited presence of a third (middle) integument (CORNER 1949; CHRISTMANN 1986) as a character that is systematically worthless at the present level of analysis, since it originates independently everywhere it occurs (*Cleistopholis*, *Cananga*, species of *Artabotrys*), but which might be useful in uniting these taxa with groups not included in the analysis. In fact, recognition of a third integument in *Tetrameranthus* (SVOMA, cited by STEINECKE 1993) means that it is a synapomorphy of at least part of the ambavioid clade. Furthermore, it occurs in *Mezzettia*, mentioned above as a possible relative of *Cleistopholis*. It is possible that trigmic seeds are a synapomorphy of the ambavioids as a whole, since the character has not been studied in *Ambavia*. The third integument of *Cananga* may also be more than an isolated occurrence, since it occurs in *Cyathocalyx*, which has a bilobed aril like *Xylopi*a and *Cananga* and thick endosperm ruminations like *Cananga* (VAN SETTEN & KOEK-NOORMAN 1992).

Position of idioblasts in the seed coat (CHRISTMANN 1987) shows a fairly coherent pattern (Fig. 17). The primitive state, retained in *Anaxagorea*, is presence of idioblasts in the inner integument, as in Myristicaceae and *Eupomatia*, but idioblasts are lost above this, in the common ancestor of the ambavioids and the remaining taxa. Idioblasts reappear in the inner integument in *Polyalthia longifolia*, in the outer integument in *Unonopsis*. Reappearance of idioblasts in the nucellus is an

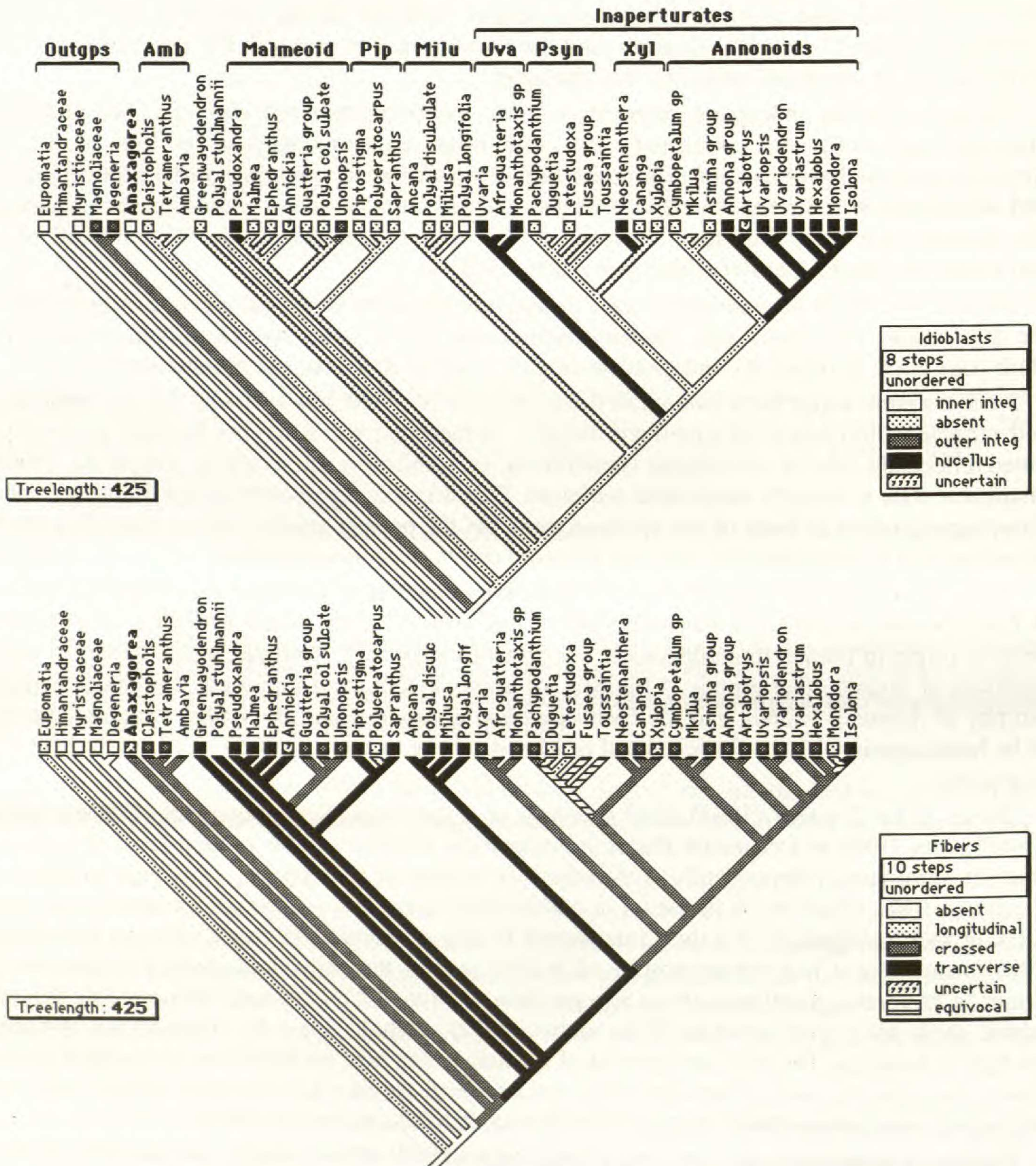


Fig. 17. — Representative most parsimonious tree (island B), showing evolution of idioblasts in the seed coat (above) and mesotesta fibers.

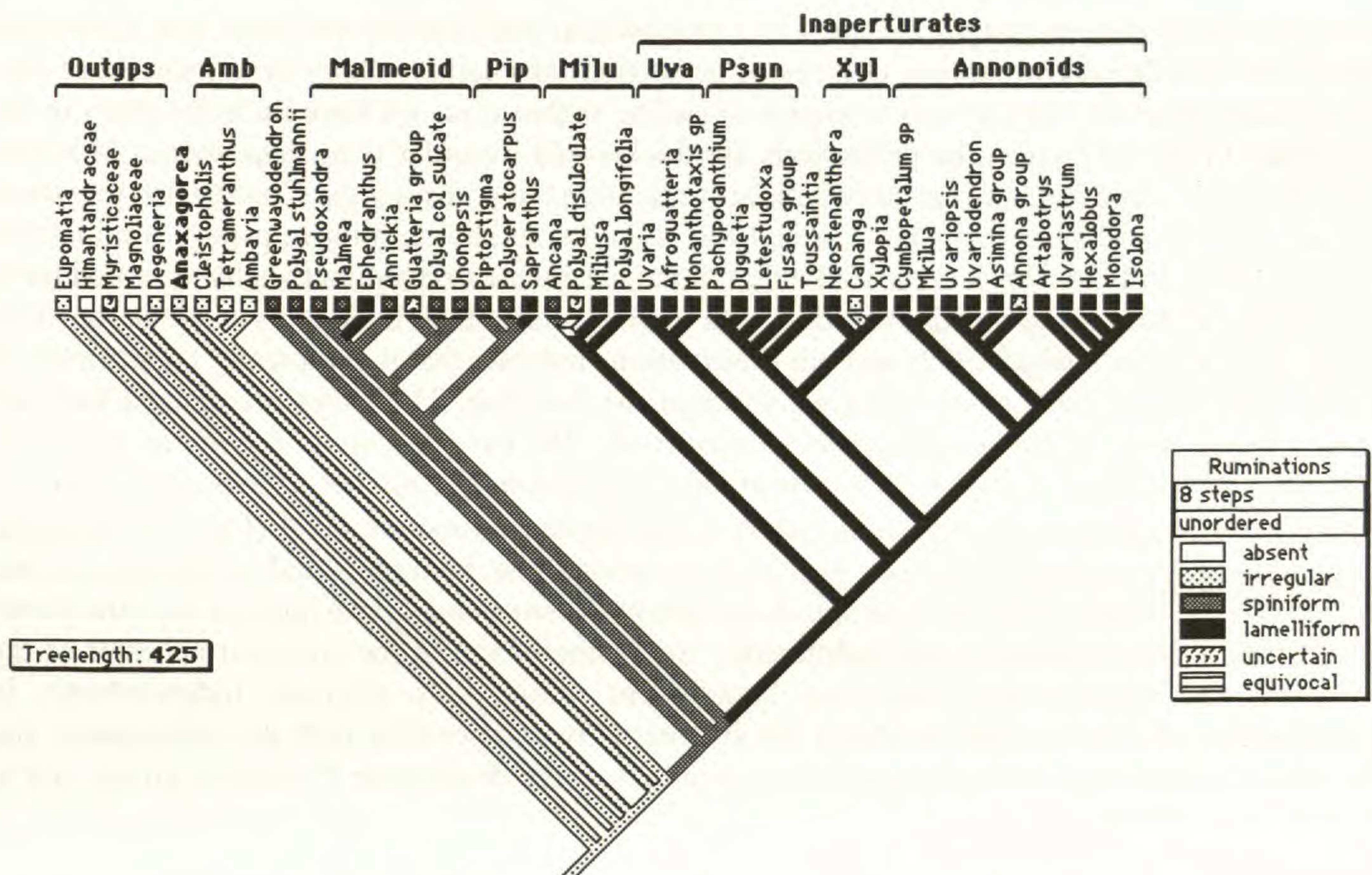


Fig. 18. — Representative most parsimonious tree (island A), showing evolution of endosperm ruminations.

important synapomorphy of the uvarioids and of all annonoids above the *Asimina-Mkilua-Cymbopetalum* clade in island B, but there are isolated origins in *Pseudoxandra* and *Neostenanthera*.

Mesotesta fibers (Fig. 17) have been cited as evidence for a relationship between *Eupomatia* and Annonaceae (CORNER 1976), but this is not borne out by our analysis, where the two taxa are separated (it is suggestive that the fibers of *Eupomatia* are longitudinal, not crossed, as in basal Annonaceae, nor transverse, as in most others). The appearance of crossed fibers (Kreuzschichtung of CHRISTMANN 1987) is a synapomorphy of *Anaxagorea* and the rest of the family, retained through the ambavioids. A shift to transverse fibers occurs below *Greenwayodendron*, but this reverses to crossed fibers in the clade consisting of *Guatteria*, *Unonopsis*, and the columellar-sulcate *Polyalthia* group (with or without *Annickia*, which is mixed), and in the inaperturates (with a reversal in *Isolona*). Longitudinal fibers arise independently in *Polyceratocarpus*, *Monodora*, and the uvarioid pseudosyncarps *Letestudoxa* and *Duguetia*, where they are equivocally homologous (*Pachypodanthium* has crossed fibers; *Fusaea* and *Toussaintia* are unknown).

Endosperm ruminations are a distinctive feature of several families of Magnoliales, but they are especially diverse in Annonaceae. The most extreme conditions are spiniform (with testal spines

often penetrating the endosperm like pins in a pincushion) and lamelliform (with four transverse plates), but lamellae show varying degrees of dissection into narrow plates or spines. Since dissected plates generally coexist with complete lamellae within taxa, we have included them in the same state. In the outgroups, the projections are thicker and irregular (e.g., *Eupomatia*: MOHANA RAO 1983), and some Annonaceae have similar ruminations (e.g., *Cananga*: CORNER 1949), where they are often described as peg-like. *Anaxagorea* is unique in having hollow, papery ruminations (CORNER 1949), but their form is most like that in the irregular category, and we have scored them accordingly. MOHANA RAO (1982), working with sections, described *Asimina* as irregular, but VAN SETTEN & KOEK-NOORMAN (1992) and our observations indicate that it is typically lamelliform; it may be that his sections passed obliquely through the lamellae. The states recognized here are actually better observed by breaking seeds transversely. We have rescored *Malmea* as spiniform rather than lamelliform, based on its narrower circumscription by CHATROU (1996, pers. comm.).

Ruminations appear to be very informative at the higher taxonomic level (Fig. 18). Irregular ruminations, as other Magnoliales, are primitive in Annonaceae, being retained in *Anaxagorea* and the ambavioids. Ruminations become spiniform below *Greenwayodendron* (and are thus the dominant pattern in piptostigmoids and malmeoids), then lamelliform in the common ancestor of the miliusoids and inaperturates. However, lamelliform ruminations originate independently in *Ephedranthus* and *Sapranthus* (evidence for its alternative association with the miliusoids), and they revert to spiniform in *Ancana* and some members of the disulculate *Polyalthia* group, and to irregular in *Cananga*.

Cytology

76. Chromosome number (0) $n = 7$, (1) $n = 8$, (2) $n = 9$ (ordered).

77. Nucleotype (0) *Tetrameranthus* type and intermediate conditions, (1) prochromosomal nucleus, chromosomes condensing proximally to distally.

78. Centromeres (0) mostly (sub)metacentric, (1) acro- or telocentric.

79. Chromosome size distribution (0) continuous, (1) bimodal.

WALKER (1972a), OKADA & UEDA (1984), MORAWETZ & WAHA (1985), MORAWETZ (1986a, 1986b), MORAWETZ & LE THOMAS (1988).

In previous analyses, we scored *Asimina* as $n = 9$, and this number was a synapomorphy of *Asimina* and the *Cymbopetalum* group, but according to MORAWETZ (pers. comm.) previous counts were in error and the correct number is $n = 8$.

The present analysis (Fig. 19) confirms our previous conclusion that the basic chromosome number in Annonaceae is $n = 8$, seen in the basal genus *Anaxagorea*, as proposed by OKADA & UEDA (1984) and MORAWETZ (1986a), rather than $n = 7$, as argued by WALKER (1972a). A decrease to $n = 7$ unites the ambavioids. Data on *Greenwayodendron*, still unknown cytologically, could test whether this genus belongs above the ambavioid clade or in it, as in previous analyses. Independent decreases occur in the *Guatteria* group (assuming its unique number $n = 14$ is derived from $n = 7$ by polyploidy: MORAWETZ & WAHA 1985) and the *Annona* group (one of its synapomorphies). Parallel increases to $n = 9$ unite (1) *Unonopsis* and the columellar-sulcate *Polyalthia* group and (2) the miliusoids, besides occurring in *Sapranthus* (other piptostigmoids are unknown).

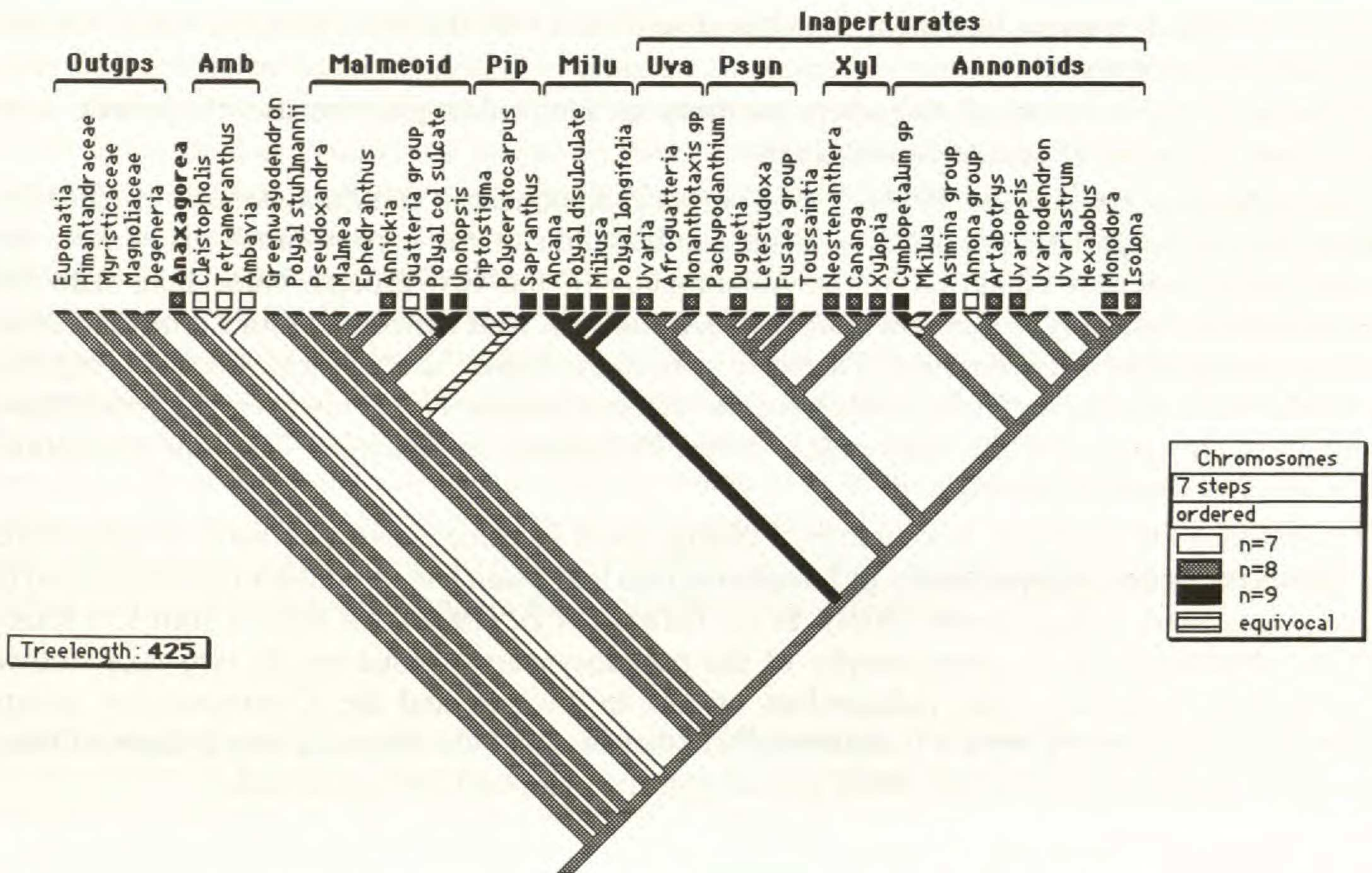


Fig. 19. — Representative most parsimonious tree (island B), showing evolution of chromosome number.

and the *Cymbopetalum* group (*Mkilua* is unknown). A weakness of this scheme is the paucity of published counts in the malmeoids and piptostigmoids. In our previous analyses, in which relationships among the malmeoids were different, $n = 9$ was a synapomorphy of the malmeoids, piptostigmoids, and miliusoids. If other malmeoids have $n = 9$, like *Unonopsis* and the columellar-sulcate *Polyalthia* group, this would add a step to our most parsimonious trees. Then trees such as Fig. 5C, in which *Annickia* is associated with *Artabotrys* and the inaperturates (as in DOYLE & LE THOMAS 1995), would become equally parsimonious, and the basic state for groups above the ambavioids would be equivocal ($n = 8$ or $n = 9$).

It must be emphasized that our conclusions on original number are based entirely on the rooting of Annonaceae inferred from other characters, since none of the other Magnoliales have numbers found in Annonaceae, and we therefore scored them as unknown. It could be argued (SCHATZ pers. comm.) that outgroup comparison is more consistent with the view that $n = 9$ is primitive, since this is closer to the numbers in *Eupomatia* ($n = 10$) and Himantandraceae and *Degeneria* ($n = 12$) (EHRENDORFER 1976), and since aneuploid reduction in chromosome numbers is more likely than aneuploid increase. Numbers in Magnoliaceae ($n = 19$) and Myristicaceae ($n = 19$ or 21) may be paleopolyploids based on $n = 9$ or 10 . This argument might in turn cast doubt on the basal position of *Anaxagorea*. As an experiment to test this hypothesis, we analyzed the data set with all outgroups rescored as $n = 9$. This had no effect on either the position of *Anaxagorea* or scenarios

for chromosome evolution: it yielded the same trees found with the basic data set, and $n = 8$ was still basic in Annonaceae.

Patterns in other cytological characters are more ambiguous, largely because of sporadic sampling (only 18 out of 42 taxa of Annonaceae).

The nucleotype (MORAWETZ 1986a, 1986b) of most Annonaceae and *Eupomatia* is prochromosomal, defined in part by proximal to distal condensation of the chromosomes. However, our results imply that this condition is not homologous in the two groups, since taxa with the *Tetrameranthus* nucleotype (Himantandraceae, *Degeneria*), with uniform chromosome condensation, are interpolated between them. The basic state in the family is equivocal, since *Anaxagorea* is prochromosomal but the ambavioids have the *Tetrameranthus* type. However, the prochromosomal type is basic for the vast clade above *Polyalthia stuhlmannii*. The one exception, *Uvariopsis*, is clearly a reversal.

Considering chromosome morphology, a change from (sub)metacentric to acro- or telocentric chromosomes occurs independently in *Tetrameranthus* and *Guatteria*. A continuous distribution of chromosome sizes is basic in the family. In LE THOMAS et al. (1994), we listed a shift to a bimodal size distribution as a synapomorphy of the pseudosyncarps, based on the two taxa studied (*Fusaea* and *Duguetia*), with independent origins in *Xylopi*a and the *Cymbopetalum* group. However, on the present trees it is equivocal whether bimodal chromosome size is derived three times or is the basic state for the whole pseudosyncarp-xylopioid-annonoid clade.

CONCLUSIONS

Despite the changes in our present results from those of our previous analyses (DOYLE & LE THOMAS 1994, 1995), closer examination shows many consistent features. In all our analyses, the basal line is *Anaxagorea*, followed by the ambavioids. Most major clades and other key relationships have also remained intact: the piptostigmoids, miliusoids, xylopioids, and pseudosyncarps, the association of *Artabotrys* with the annonoids, and the group consisting of *Monodora*, *Isolona*, and *Hexalobus* (cf. WALKER 1971a). Most groups that are monophyletic in some analyses but not in others, such as the malmeoids, annonoids, and inaperturates, are at least paraphyletic when they are not monophyletic (i.e., the same groups of taxa are rooted and attached to each other differently), rather than polyphyletic; an exception is the former uvarioids, now broken into two well-separated clades. Many of the instabilities are due to “jumping” of a limited number of insecurely placed taxa (*Annickia*, *Guatteria*, *Sapranthus*, *Ancana*, *Toussaintia*, *Artabotrys*). As should be expected, since parsimony analysis is a method of searching for the global hypothesis most consistent with the totality of available characters, most relationships found have been suggested by previous authors working on one or another character set. Furthermore, major aspects of the tree, especially the rooting near *Anaxagorea* and the existence of the inaperturate clade, are confirmed by preliminary molecular analyses (VAN ZUILEN 1996; BYGRAVE & CHASE pers. comm.).

This study has also identified characters and taxa that are in special need of study, and which might help resolve some of the uncertainties. For example, statistical analysis of many somewhat

dubious, semi-quantitative characters, as in wood anatomy, might lead either to definition of more defensible breaks between states or to elimination of characters entirely. Many characters that have been studied well in one geographic area or subgroup of the family should be investigated on a world scale, such as position of prophylls (which will require observation of living plants), leaf anatomy, and cytological characters. Analyses of internal relationships in large, variable taxa, as done for the *Cymbopetalum* group by JOHNSON & MURRAY (1995), should help in reconstructing basal states, which may reduce the number of uncertain character scorings (e.g., in the *Annona* group, *Artabotrys*, the *Monanthotaxis* group, and *Polyalthia* and its relatives in Asia). Pollen ultra-structure work could also help integrate possibly critical Asian taxa into the analysis, as noted in discussion of *Mezzettia* and *Monocarpia*. Given the high level of morphological homoplasy in Annonaceae, it may be that only molecular analyses will be capable of resolving higher-level relationships. However, even if molecular data are found to be both necessary and sufficient for unraveling the phylogenetic topology, analysis of morphological characters along the lines presented here will be necessary in order to plot morphological changes on molecular trees and to address biologically interesting questions on the evolution of these plants.

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An extraordinary saprophyte in the genus *Habenaria* (Orchidaceae) from Madagascar

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Summary: *Habenaria saprophytica*, from Madagascar, is described here. It is the first saprophytic species known in the genus.

Résumé : Description de *Habenaria saprophytica*, de Madagascar. C'est la première espèce saprophyte connue pour le genre.

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While examining recent collections of orchids at Paris, Kew, and in the Herbarium of the Parc Tsimbazaza in Antananarivo, Madagascar, we found a strange, achlorophyllous orchid which appeared to be a saprophytic *Habenaria*.

The large genus *Habenaria* is cosmopolitan with the majority of species tropical. It is particularly well represented in tropical and South Africa, the tropical Americas, and eastern Asia. In Madagascar about 25 species have so far been described. So far all known species of *Habenaria*, and segregate genera such as *Roeperocharis*, *Centrostigma*, *Bonatea* and *Platycoryne*, have been autotrophic plants with green leaves. Moreover, no saprophytic species have been identified in the subtribe Habenariinae. The present species is therefore a strange one, unique in the genus *Habenaria* where its floral morphology seems to place it.

Saprophytic species have been reported for a number of genera in which the majority of species are autotrophic, for example, *Eulophia* (*E. galeoloides* Kraenzl., *E. bletilloides* Schltr. and for Madagascar *E. hologlossa* Schltr. and *E. mangelotiana* Bosser & Veyret), *Brachycorythis* (if *Schwartzkopffia* is included), *Cystorchis* (*C. aphylla* Ridl.), and *Tropidia* (*T. saprophytica* J.J. Sm.). In *Epipactis*, one species, the European *E. purpurata*, is occasionally achlorophyllous.

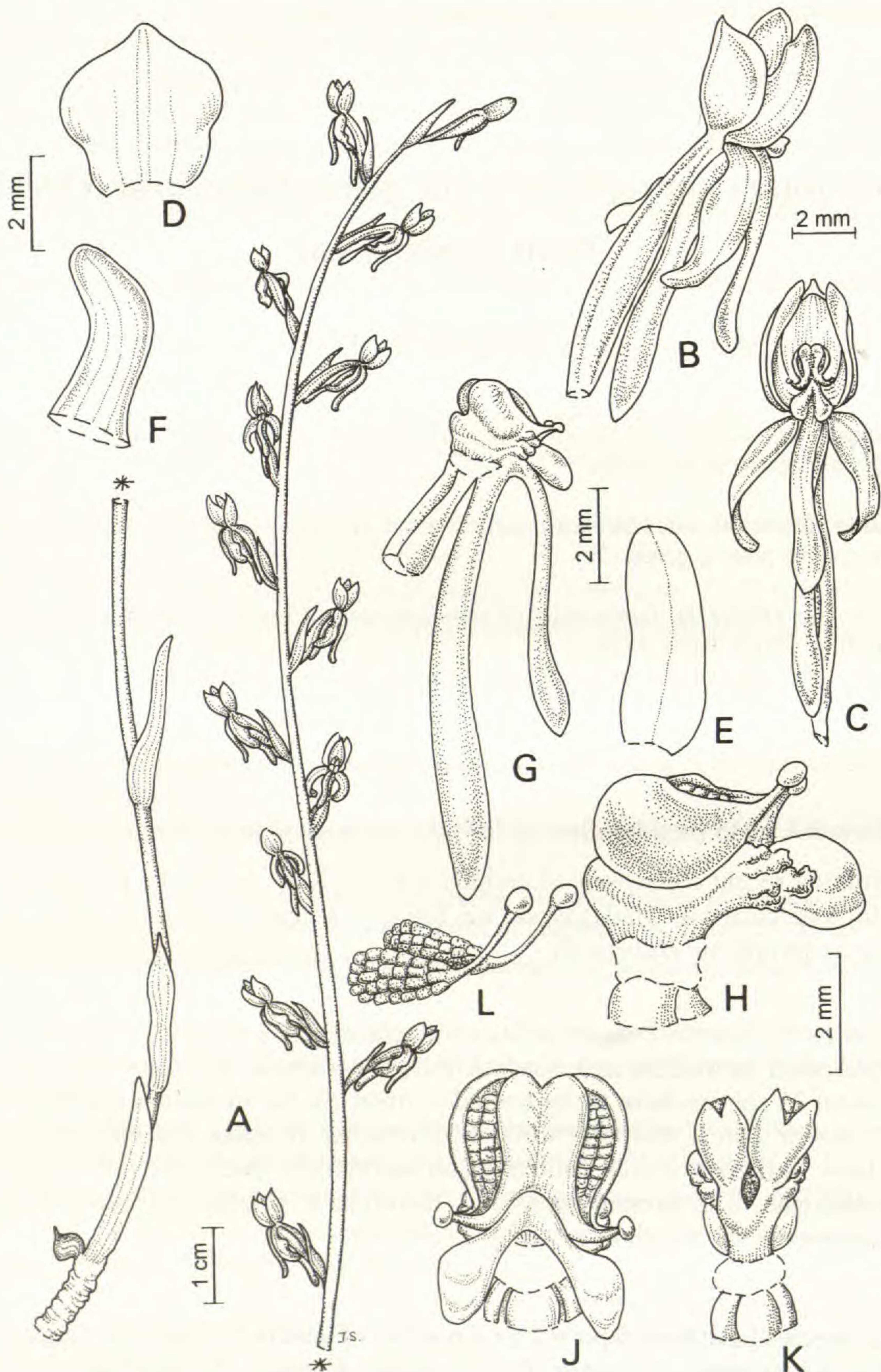


Fig. 1. — *Habenaria saprophytica*: A, habit; B, flower side view; C, flower front view; D, dorsal sepal; E, lateral sepal; F, petal; G, column, lip & spur; H, column side view; J, column front view; K, column from above; L, pollinia. All drawn by Judi STONE from the type specimen.

Habenaria saprophytica Bosser & P.J. Cribb, **sp. nov.**

Species notabilis in genere. Ab speciebus aliis planta saprophytica aphylla, floribus albis, petalis falcatis simplicibus, labello lineari-oblongo, pendulo, rostello brachiis clavatis brevibus lobo medio triangulare quam loculis antherae brevioribus bene diversa.

TYPUS. — *Malcomber & Rapanarivo 1214*, Madagascar, 35 km SW of Antsiranana, Mt. d'Ambre National Park, N of Grande Cascade, Forêt d'Ambre, 18 Feb. 1992 (holo-, P! ; iso-, K!, MO!, TAN!), only known material.

A small, achlorophyllous, saprophytic, aphyllous, glabrous, terrestrial herb 8-30 cm tall, white in all its parts; subterranean parts unknown. Stem terete, erect, bearing sterile 1-2 cm long sheaths in lower half of stem, each shorter than a node. Inflorescence racemose, up to 15 cm long, laxly 3- to 14-flowered; bracts lanceolate, acuminate, 5-15 mm long, more or less equalling the pedicel and ovary in length. Flowers white, 10-12 mm long; pedicel and ovary terete, 10-11 mm long. Dorsal sepal hooded subcircular, rounded at the apex, 2.5-3.5 mm in diameter, one-nerved; lateral sepals deflexed, somewhat oblique-falcate, oblong-lanceolate, obtuse, $3.2-5.5 \times 2$ mm, one-nerved. Petals slightly fleshy at the apex, falcate, oblong-elliptic, obtuse, slightly auriculate at base in front, $3.2-4 \times 1.5-2$ mm, 2-nerved. Lip fleshy, longitudinally convex, dependent, entire, linear-oblong, obtuse or rounded at apex, $4.5-6 \times 0.7-0.8$ mm; spur cylindric-fusiform or narrowly fusiform, 8-9 mm long, more or less parallel to ovary then slightly incurved in apical half. Column 2.5-3 mm long; stigmatic arms clavate, deflexed, 1 mm long; rostellum 3-lobed, side lobes 0.7-0.8 mm long, midlobe triangular, much shorter than the anther loculi; staminodes entire, deflexed, very short.

DISTRIBUTION AND HABITAT. — Madagascar; known only from the type collection. Terrestrial in lower montane forest, alt. 610 m.

We have assigned this strange orchid to the genus *Habenaria* because of its free stigma lobes, and rostellum with a very short triangular midlobe much shorter than and appressed to the anther loculi. However, its entire lip, petals and sepals are reminiscent of the Africo-Madagascan genus *Platycoryne*, itself a segregate of *Habenaria*. The bright orange-flowered *Platycoryne pervillei* is the only species recorded from Madagascar.

The ghost-like appearance of this leafless orchid sets it apart from all other Madagascan *Habenaria* species, and it is most likely to be confused with the unrelated *Epipogium roseum* which has pendent flowers with an ovate, callose lip and short spur, much shorter than the lip.

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